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Dedications

With the help of the Almighty, I was able to complete this thesis that I dedicate:

In memory of my grandparents, I can never thank you enough for all the exemplary support and exceptional love that you have given me since I was little.

May God reserve for you a place among the best in paradise and may this work be a prayer for you.

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List of abbreviations

A: Absorbance

***A. baumannii*:** *Acinetobacter baumannii*

AB: *Acinetobacter baumannii*

AHL: Acyl Homoserine Lactone

AIP: Auto-Inducing Peptide

AL: *Acinetobacter lwoffii*

AMPs: Anti-Microbial Peptides

BBC: Biofilm Bactericidal Concentration

BEA: Bile Esculin Agar

BHI: Brain Heart Infusion

BLAST: Basic Local Alignment Search Tool

bp: bases pair

BSI: Bloodstream Infection

CA-BSI: Catheter Associated Bloodstream Infection

CA-UTI: Catheter Associated-Urinary Tract Infection

CBE: Cyto-Bacteriological Examination

c-di-GMP: Cyclic Dimeric Guanosine Monophosphate

CFU: Colony Forming Unit

CLA-BSIs: Central Line-Associated Bloodstream Infections

CLSI: Clinical and Laboratory Standards Institute

CRA: Congo Red Agar

CVC: Central Venous Catheter

DMSO: DiMethylSulfOxyde

dNTPs: DeoxyNucleotides TriPhosphate

EC: *Escherichia coli*

***E. coli*:** *Escherichia coli*

eDNA: Extracellular DNA

EF: *Enterococcus faecalis*

EMB: Eosine Methylene Blue

***En. faecalis*:** *Enterococcus faecalis*

EPS: Exopolysaccharides

ESBL: Extended Spectrum β-Lactamases

EUCAST: European Committee on Antimicrobial Sensitivity Testing

FBEC: Fractional Biofilm Eradication Concentration

HAI: Hospital-Acquired infections

IAD: Intra-Abdominal Drain

ICU: Intensive Care Unit

IVC: Intravenous Catheter

***K. pneumoniae*:** *Klebsiella pneumoniae*

KP: *Klebsiella pneumoniae*

LC: Lethal Concentration

LPS: Lipopolysaccharide

MBC: Minimal Bactericidal Concentration

MBEC: Minimal Biofilm Eradication Concentration

MDR: Multi-Drug Resistant

MIC: Minimal Inhibitory Concentration

MR: Methyl Red

MRS: de Man Rogosa and Sharpe

List of abbreviations

MSA: Mannitol Salt Agar

NA: Nutrient Agar

NB: Nutrient Broth

NI: Nosocomial Infection

OD: Optical Density

***P. aeruginosa*:** *Pseudomonas aeruginosa*

PBS: Phosphate Buffered Saline

PCR: Polymerase Chain Reaction

PIA: Polysaccharide Intercellular Adhesin

PVC: Peripheral Venous Catheter

PVC-BSIs: Peripheral Venous Catheter-Associated Bloodstream Infections

QS: Quorum Sensing

RBC: Red Blood Cells

rDNA: Ribosomal DeoxyriboNucleic Acid

RNA: RiboNucleic Acid

RP-HPLC: Reverse Phase High Performance Liquid Chromatography

SAAP: Synthetic Antimicrobial and Anti-biofilm Peptide

***S. aureus*:** *Staphylococcus aureus*

***S. epidermidis*:** *Staphylococcus epidermidis*

SDS: Sodium Dodecyl Sulfate

ST: *Staphylococcus*

Taq polymersae: *Thermus aquaticus* Polymerase

TCP: Tissue Culture Plate

TS: Tryptone Salt

TSA: Trypticase Soy Agar

TSB: Trypticase Soy Broth

TSI: Triple Sugar Iron

URC: Urinary Catheter

UV: Ultra Violet

VP: Voges Prauskauer

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General introduction

Every year, millions of catheters are implanted by health services to improve the management of acute and chronic diseases in adult and pediatric patients **(Ullman *et al*., 2015; Santos *et al*., 2020; Zhang *et al*., 2020)**. Unfortunately, their use inevitably allows patient's own flora or accidental environmental contaminants to access the underlying tissues and cause fatal complications **(Guenezan *et al*., 2018; Casimero *et al*., 2020, Aumeran *et al*., 2021)**. About 60 to 70% of nosocomial infections (NI) are linked to medical devices **(Yasir *et al*., 2018)**, and several microorganisms such as *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus faecalis* are widely implicated in these infections **(Cortese *et al*., 2018; Dadi *et al*., 2021)**. These bacteria may originate from the endogenous flora of the patients (e.g. from mucosa) or from exogenous sources, such as other patients, healthcare workers, healthcare settings surfaces, or contaminated objects **(Neoh *et al*., 2017)**. The main complications result from the ability of microorganisms to adhere to surfaces and form biofilms **(Wi and Patel, 2018)** and the presence of persister cells **(Wang *et al*., 2023)** thereby making the antibiotic treatment ineffective and biofilm eradication impossible **(Wainwright *et al*., 2021; Theis *et al*., 2023)**.

In fact, catheter- associated bloodstream infections (CA-BSIs) are an important cause of hospital-acquired infections originating from an intravenous catheter (IVC) and associated with morbidity, mortality, and hospital cost **(Gahlot *et al*., 2014)**. Central venous catheters (CVCs) are among the most widely used medical devices in critically ill patients. However, central line-associated bloodstream infections (CLA-BSIs) are the most common complications which are usually associated with the use of these CVCs **(Guenezan *et al*., 2018)**, causing an increase in the rate of morbidity and mortality in health establishments as well as the length and costs of the stay **(Pitiriga *et al*., 2020)**. In fact, 80,000 CVC-associated bloodstream infections (CVC-BSIs) mainly occur in intensive care units (ICUs) with a death rate of 12 to 25% **(Zander and Becker, 2018)**. Concerning peripheral venous catheters (PVCs), it has been estimated that 30 to 80% of hospitalized patients have a PVC in place during their hospitalization **(Sato *et al*., 2017)** and more than a billion PVCs are used each year around the world **(Beecham and Tackling, 2021)**. Among the side effects observed when a PVC is used are the following: phlebitis, partial dislodgement, accidental removal, occlusion, infiltration (fluid moving into surrounding tissue), and rarely infections **(Miliani *et al*., 2017; Chen *et al*., 2021)**. The incidence of BSIs associated with peripheral venous catheters (PVC-BSIs) is generally low, with a rate of 0.1% of short catheters inserted (0.5 episodes per 1000 days of intravascular catheter) **(Bertoglio *et al*., 2017)**, unlike the incidence of CVC-BSIs which is 2.7 episodes per 1000 days of intravascular catheter

(Sato *et al*., 2017). Moreover, IVC can be easily colonized by pathogenic microorganisms which lead to the formation of biofilms, other potential sources of BSIs **(Chauhan *et al*., 2014)**. Additionally, 81% of all vascular catheters that were placed *in situ* for 1–14 days were reported to be colonized by bacteria originating from biofilm **(Richards *et al*., 2014)**. Biofilm colonization of IVCs continues to affect healthcare settings **(Donlan *et al*., 2014)**. Several factors increase the risk of catheter infections such as patient immunodeficiency, length of prolonged catheterization, catheter material, anatomical site of catheter insertion, poor hygiene, poor catheter insertion, and handling methods **(Lutwick *et al*., 2019)**.

Similarly, catheter-associated urinary tract infections (CA-UTIs) are the most commonly hospital-acquired infections worldwide **(Cortese *et al*., 2018)** and represent up to 40% of NIs. This is not surprising since 70% of UTIs are associated with urinary catheters (URC) and approximately 20% of hospitalized patients have a URC, especially those in ICUs **(Melo *et al*., 2016)**. Despite the high risk of acquiring infections with multidrug-resistant (MDR) opportunistic pathogens, most cases of catheter-associated bacteriuria are asymptomatic. However, when an episode of CA-UTI becomes symptomatic, the resulting sequelae can range from mild (fever, urethritis, and cystitis) to severe (catheter encrustation, bladder stones, pyelonephritis, endotoxic shock, and bacteremia). Left untreated, these infections can lead to urosepsis and death **(Jordan *et al*., 2015; Adegun *et al*., 2019)**. Indeed, for each day that a URC is *in situ*, there is a 3–8% incidence of bacteriuria, and in the majority of cases, long-term catheterization results in continued bacteriuria and symptomatic CA-UTI **(Gaston *et al*., 2020)**. Several factors increase the risk of CA-UTIs, such as female gender, older age (i.e., age>50 years old), diabetes mellitus, faecal incontinence, immunocompromised status and healthcare factors such as lack of systemic antibiotics, catheter insertion outside the operating room, prolongation of the duration of catheterization, and poor quality of catheter care **(Haque *et al*., 2018; Anggi *et al*., 2019; Crader and Leslie, 2021)**. CA-UTI is linked with biofilm formation along the surface of the catheter **(Gaston *et al*., 2020)**. Indeed, the presence of a URC facilitates bacterial colonization due to the development of a conditioning film of host proteins which provides bacteria with an ideal substrate for fixation **(Loose *et al*., 2021)**.

In view of all this, novel antimicrobial and anti-biofilm/persister agents for the treatment of catheter-associated infections are urgently required. Antimicrobial peptides (AMPs), which are molecules of the first line of defense against infections **(Chernysh *et al*., 2018)**, are considered as promising candidates as they combine broad-spectrum antimicrobial activities with

immune-modulating capabilities **(de Breij *et al*., 2018; Van der Does *et al*., 2019; Kim *et al*., 2023b)**. Interestingly, these peptides kill bacteria by mechanisms different from those of current antibiotics, such as interacting with and subsequently disrupting the microbial plasma membrane. Moreover, some AMPs can interact with the extracellular polysaccharides of the matrix, leading to the disintegration of biofilms **(Di Luca *et al*., 2014; Jaśkiewicz *et al*., 2018)**. One of the best-studied AMPs is human cathelicidin LL-37 **(Jaśkiewicz *et al*., 2018; Spencer *et al*., 2018)**, from which the LL-37-based synthetic antimicrobial and anti-biofilm peptide (SAAP)-148 was developed using random amino acid substitutions in the C-terminal part of LL-37 **(de Breij *et al*., 2018)**. In the study of **de Breij *et al.* (2018)**, the peptide SAAP-148 was highly efficient in killing a panel of planktonic MDR- bacteria, including colistin-resistant *E. coli*, *K. pneumoniae*, and *A. baumannii*. Further studies have also shown the efficacy of this peptide against biofilms **(de Breij *et al*., 2018; van Gent *et al*., 2022)** and persister cells **(de Breij *et al*., 2018; Scheper *et al*., 2021; Verheul *et al*., 2021)**.

Recently, using computer-assisted deep learning discovery approaches (at the Drug Repurposing Hub) **(Stokes *et al*., 2020)**, halicin was identified as the first-in-class novel small molecule based on the probability of being able to inhibit *E. coli* growth. Importantly, additional assays demonstrated that halicin exhibits broad-spectrum antibacterial activities against clinical strains, such as carbapenem-resistant *Enterobacteriaceae,* MDR- *A. baumannii* and *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, and *Clostridium difficile, in vitro* and in murine infection model **(Stokes *et al*., 2020)**. Likewise, this agent was demonstrated as being effective against MDR- *A. baumannii* 3086 **(Booq *et al*., 2021)** and 24- h immature and 7-days mature biofilms formed on a polypropylene plate **(van Gent *et al*., 2022)**. Moreover, it was suggested that the compound kills bacteria by dissipating the transmembrane electrochemical gradient, ΔpH, after binding with iron **(Aburayan *et al*., 2020; Stokes *et al*., 2020)**.

These antimicrobial agents, SAAP-148 and halicin are under investigation at the laboratory of the department of infectious diseases of the Leiden University medical center (LUMC), where the study was undertaken, and it constitutes a contribution to better understanding of the antimicrobial, anti-biofilm and anti-persisters properties of these agents alone and in combination.

In Algeria, until now, there are few publications dealing with catheter- associated biofilm bacteria and none report in Bejaia city. Furthermore, to our knowledge this is the first report on

novel approaches, using AMPS or halicin, to combat catheter-associated biofilms in Algeria. This lack of information has attracted our attention and we have initiated this project which concerns the study of the biofilm formation potential of bacterial strains isolated from different medical devices and their possible eradication by the peptide SAAP-148 and halicin.

The main objectives of this research project are as follows:

- Characterization of pathogenic strains (including opportunistic pathogens), isolated from medical devices, on their biofilm formation potential and surface properties (hydrophobicity and aggregation) and pathogenicity (hemolytic activity and virulence genes content).

- Knowledge of the resistance profile of the isolated pathogens towards the widely used antibiotics in our healthcare settings in the purpose to better manage the antibiotics administration.

- Exploration of new alternative treatment strategies for prevention and/or eradication of biofilms and persistent cells by antimicrobial agents such as antibacterial peptides (SAAP-148) and halicin, alone or in combination.

The present document is divided into two main parts:

- The first part is deserved for a literature review to shed the light on the background available on the subject of the study. The first chapter describes biofilms, their different steps of formation, factors influencing their formation, and their resistance to antibiotics. The second chapter is devoted to medical device-associated biofilm infections and the third chapter to the most bacteria involved in these infections. On the other hand, the antimicrobial peptides, as new anti-biofilm and anti-persisters agents, are described in the fourth chapter.

- The second part is dedicated to the presentation of the experimental protocols and the obtained results followed by a deep confrontation with the literature data.

Finally, the main conclusions and driven outlooks are then presented at the end of the document.

Literature review

Chapter I. Biofilm: an incredible bacterial way of life

1. History

Microorganisms possess the remarkable capacity to live in nearly any type of environment while utilizing intricate survival mechanisms to protect themselves against harsh conditions **(Di Somma *et al*., 2020b)**. Bacteria can be free in planktonic form or adhered to biotic or abiotic surfaces and aggregate forming microbial communities surrounded by an extracellular matrix called biofilm **(Verderosa *et al*., 2019)**. In the 17th century, Anthony van Leeuwenhoek made the initial discovery of biofilms on his own dental plaque **(Høiby, 2014)**. In 1933, Henrici observed using microscope, that microorganisms in fresh water adhere to submerged surfaces and do not remain free floating **(Høiby, 2017)**. In 1943, Zobell and Allen studied the bacterial adhesion and its growth on glass slides submerged in seawater **(Høiby, 2017)** and observed that the number of microorganisms was greater on the slides than in seawater. In 1978, Costerton described that the biofilm is composed of a complex of microorganisms embedded in an extracellular matrix and adhered to surfaces **(Colombo and Tanner, 2019)**. Finally, in 2002, Donlan and Costerton defined a biofilm as: "a sessile microbial community irreversibly attached to a substrate or interface and surrounded by a polymeric extracellular matrix produced by themselves" **(Donlan and Costerton, 2002)**. Since then, several studies on biofilms have continued to develop, whether in the medical or industrial field. Indeed, over the past three decades, biofilms have attracted a lot of interest, as it is clear that biofilm formation is the predominant microbial lifestyle in the environment **(Bisht and Wakeman, 2019; Penesyan *et al*., 2021)**.

Biofilm is a serious problem in different fields and cause acute issues in clinical settings and industry **(Srinivasan *et al*., 2021)**. Bacterial biofilms are able to adhere to multiple surfaces such as medical devices including venous and urinary catheters, prosthetic joint and heart valves causing biofilm-related infections. Moreover, biofilms act as protection barrier against antimicrobial agents thereby leading to therapeutic failure and increased mortality and morbidity rates **(Kannappan *et al*., 2017; Zhang *et al*., 2020; Li *et al*., 2023)**. The bacterial biofilm is 10 to 1000 times more resistant to antimicrobial agents compared to planktonic bacteria **(Sharma *et al*., 2019; Uruèn *et al*., 2020)** and can escape the host immune responses **(Cangui-Panchi *et al*., 2023)**. In addition, biofilms protect bacteria from harsh conditions such as nutrient deprivation, temperature and pH variations, radiation, extreme salinity, etc., **(Yin *et al*., 2019; Srinivasan *et al*., 2021)**.

2. Biofilm composition

A biofilm is composed of microorganisms (5-35% of biofilm volume) adhered to a surface and embedded in an extracellular polymeric matrix. This matrix is mainly composed of proteins (>2%), exopolysaccharides or EPS (1-2%) (Table 1), extracellular DNA or eDNA (<1%) and RNA (<1%). Furthermore, this matrix is extensively hydrated and comprises up to 97% water, which plays a crucial role in the transport of nutrients throughout the biofilm **(Jamal *et al*., 2018; Karygianni *et al*., 2020)**.

The presence of water provides to the biofilm a flexible and viscous structure allowing the movement of bacterial cells in the matrix **(Pinto *et al*., 2020)**. The EPS **(Table 1, Figure 1)** in the matrix allow bacterial aggregation and their adhesion to surfaces **(Karygianni *et al*., 2020)**, provide structural stability to the biofilm **(Mishra *et al*., 2023)**, access to nutrients, adaptation to environmental changes and survival. They also act as a barrier against antimicrobial agents and host immune responses **(Srinivasan *et al*., 2021)**. Another important matrix component is proteins which are involved in adhesion, virulence and bacterial protection towards harsh conditions **(Srinivasan *et al*., 2021)**. eDNA is also an important component, derived from chromosomal DNA which is released into the extracellular environment either by cell lysis or membrane vesicles **(Uruén *et al*., 2020)**. It plays an important role in the structural stability of the biofilm, nutrient intake, genetic transfer and resistance to antimicrobial agents **(Srinivasan *et al*., 2021)**.

3. Biofilm physiology

Bacteria are located in different areas within a biofilm creating physiological heterogeneity characterized by divergences in gene expression and metabolic activity, a source of microenvironments leading to phenotypic diversity **(Hall and Mah, 2017; Bisht and Wakeman, 2019)**. This heterogeneity is due to the presence of nutrient and oxygen gradients within the biofilm **(Uruèn *et al*., 2020)**.

In fact, bacteria found in the upper zones of the biofilm are more active due to better accessibility to nutrients and greater ease of elimination of metabolic wastes compared to bacteria located at the bottom of the biofilm **(Salgar-Chaparro *et al*., 2020)**. In biofilm, we distinguish bacteria with different metabolisms (aerobic, microaerobic and fermentative) and different growth rates (rapid growth, slow growth, dormant cells, persistent cells) **(Uruèn *et al*., 2020)**.

Table 1. Types of bacterial EPS

Microorganism	Name	Structure/Composition	Function	References
Enterobacteriaceae	Colanic acid (M antigen)	Composed of glucose, galactose, and glucuronic acid	Initial attachment to surfaces, scaffolding, cohesion, pathogenesis	**Limoli *et al*., 2015** **Benincasa *et al.*, 2016 ; Pal *et al*., 2019**
	Capsular polysaccharide (K antigen)	Composed of repeating monosaccharides joined by glycosidic linkages	Expressed in mature biofilms, aiding in the maintenance of biofilm size and dispersal	
Acinetobacter baumannii	Capsular polysaccharide (K antigen)	Composed of repeating oligosaccharide subunits (4-6 sugar)	Adhesion, scaffolding, protection against immune system and antibiotics	**Singh *et al.*, 2018b; Niu *et al*., 2020**
Staphylococcus aureus	Polysaccharide intercellular adhesin (PIA)	poly-β (1-6)-*N*-acetylglucosamine	Adhesion, cohesion, scaffolding, stability, protection against antibiotics	**Karygianni *et al*., 2020**
Enterococcus faecalis	Enterococcal polysaccharide antigen (EPA)	composed of glucose, rhamnose, *N*-acetylglucosamine (GlcNAc), *N*-acetylgalactosamine (GalNAc), and galactose	Cohesion, stability, antibiotic resistance, phagocytosis evasion	**Guerardel *et al*., 2020 ; Ramos *et al*., 2021**

4. Biofilm life cycle

Biofilm formation is complex and takes place in six stages: reversible attachment, irreversible attachment, proliferation and formation of microcolonies, multiplication and cells recruitment, then maturation, and finally detachment/dispersion of the biofilm **(Figure 1)**. Each of these steps will be detailed below.

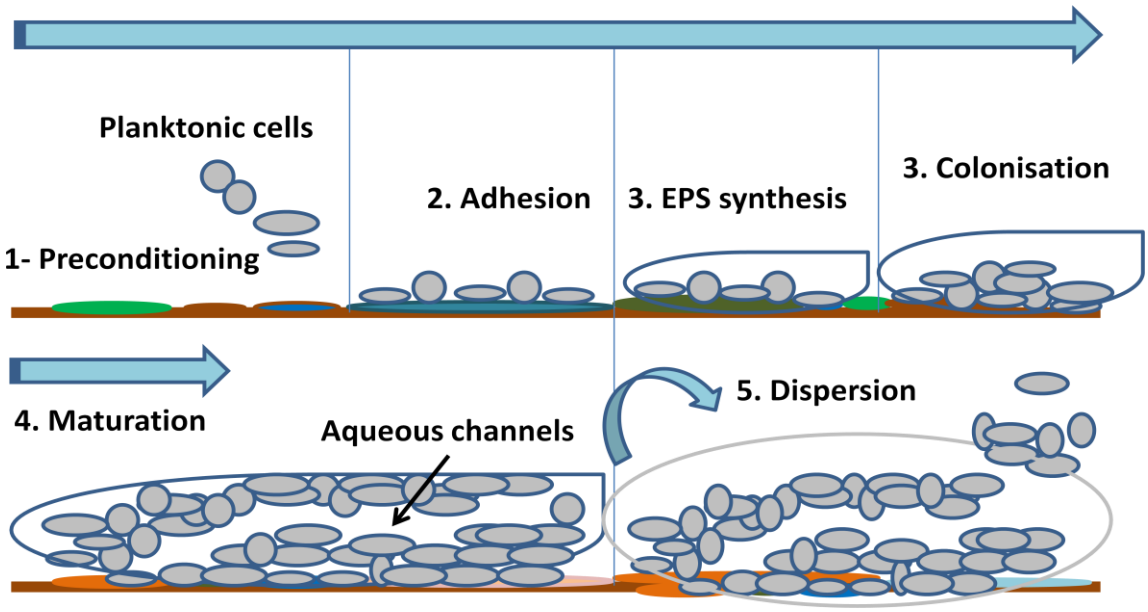


Figure 1. Process of biofilm formation **(Bouhrour *et al.*, 2024)**

4.1. Reversible attachment

The ability of bacteria to adhere to surfaces is a crucial parameter in biofilm development **(Akbar *et al.*, 2023)**. The first step of bacterial adhesion on surface is the transport of cells to the surface **(Nakanishi *et al.*, 2021)**. The initial attraction mainly involves physical interactions such as Van der Waals attractive forces, electrostatic forces, hydrophobic interactions, Brownian motion and gravitational forces **(Katsikogianni *et al.*, 2004; Nakanishi *et al.*, 2021; Ma *et al.*, 2022; Zhao *et al.*, 2023)**.

There are two types of physical interactions: long-range interactions which require a distance >50 nm between the cell and the surface thus implying a weak electrostatic interaction (Van der Waals forces); and short-range interactions including a distance <5 nm between the cell and the surface with involvement of different interactions such as hydrogen, ionic, hydrophobic bonds and bacterial adhesins inducing more stable adhesion of bacteria to the surface. These interactions are primordial for the reversible adhesion of bacterial cells to surfaces **(Armentano *et al.*, 2014; Brožková *et al.*, 2022; Oopath *et al.*, 2022)**.

During reversible attachment, the bacteria use appendages (e.g. flagella and pili) to overcome repulsive electrostatic (due to negative charge of bacterial membrane) and hydrodynamic (in liquid environment) forces; and also increase the adhesive strength thereby becoming irreversibly adhered **(Berne *et al*., 2015; Khatoon *et al*., 2018; Li *et al*., 2021b)**.

Bacterial adhesion occurs on a conditioning film formed on the surface after deposition of organic, inorganic and cellular components (fibronectin, fibrinogen, laminin and polysaccharides) found in the environment surrounding the surface, constituting a base on which the biofilm will develop **(Khatoon *et al*., 2018; Kreve and Reis, 2021; Li *et al*., 2021b)**.

The bacteria interact with the components of this surface through appendages, attractive forces or adhesins **(Kreve and Reis, 2021)**. Bacterial movement can also be induced by chemotaxis due to the presence of diffusible chemical gradients which form from various chemical stimuli or the degradation of components (e.g. aspartate, glucose and galactose) **(Cheng *et al*., 2019; Nakanishi *et al*., 2021)** thus favoring the interaction between bacteria and the surface to colonize **(Zhao *et al*., 2023)**. However, if the surface is unsuitable for bacterial adhesion, the bacteria return to the planktonic state **(Li *et al*., 2023)**.

4.2. Irreversible attachment

Irreversible adhesion is characterized by stronger interactions (distances <1.5 nm) between the bacteria and the surface such as hydrogen bonding, ionic bonding and covalent bonding, allowing bacterial adhesins to bind to the surface **(Li *et al*., 2021b; Zhao *et al*., 2023)**. Moreover, during this stage, the bacterial cells adhere to each other forming aggregates on the surface in an irreversible and more stable way **(Sharma *et al*., 2023)**. This step is characterized also by the initial production of EPS, a major constituent of the biofilm matrix which allows the bacterial cohesion and their attachment to surfaces **(Muhammad *et al*., 2020)**.

Structural and physiological changes occur through the expression of genes responsible for the production of bacterial surface structures (lipopolysaccharides or LPS, *fimbriae*, pili and capsules) to make the adhesion firmer. The repression of genes involved in bacterial motility is also triggered, thus promoting the transition to the biofilm lifestyle **(Abebe, 2020; Li *et al*., 2021b; Nakanishi *et al*., 2021)**.

4.3. Cell growth and microcolonies formation

Once bacterial cells attach to the surface, bacteria multiply rapidly and reach high cell density, leading to the activation of a second messenger (bis-(3′-5′)-cyclic dimeric guanosine

monophosphate “c-di-GMP”) and cell-to-cell communication (*Quorum sensing*) which helps in virulence and biofilm genes regulation, and EPS production **(Khatoon *et al*., 2018; Guzmán-Soto *et al*., 2021)**.

In fact, c-di-GMP molecules catalyzed through *Quorum sensing* (*QS*) repress motility appendages (e.g. flagella) and stimulate EPS production **(Li *et al*., 2021b)**. As the concentration of cells rises, bacteria glide against one another, resulting in the formation of bacterial clusters known as "immature biofilms" or microcolonies **(Guzmán-Soto *et al*., 2021)**. These microcolonies start to express different EPS, which act as “glue” in order to strengthen attachment to the surface **(Abebe, 2020; Nakanishi *et al*., 2021)**. Thus, the biofilm becomes denser and more complex **(Oopath *et al*., 2020)**.

4.4. Multiplication and recruitment of cells

At this stage, attached and aggregated bacteria continue their proliferation by multiplication and recruitment of additional surrounding planktonic cells **(Sauer *et al*., 2022)**. In addition, the multilayers continue to form, inducing the increase in thickness thereby allowing the transition from a two-dimensional arrangement to a three-dimensional arrangement **(Guzmán-Soto *et al*., 2021)**.

4.5. Biofilm maturation

During biofilm maturation, the attached multilayer cells have grown into a mature biofilm with a typical three-dimensional (3D) structure **(Srinivasan *et al*., 2021)** also called “mushroom” structure **(Muhammad *et al*., 2020; Oopath *et al*., 2022)**. Channels filled with water are formed in the biofilm allowing the transport of nutrients, signaling molecules and elimination of waste **(Jamal *et al*., 2018; Preda and Săndulescu, 2019)**.

At this step, bacterial microcolonies communicate with each other, thanks to *QS*, via signaling molecules, called autoinducers (AIs), thereby allowing the regulation of the expression of virulence genes. The production of EPS by bacterial cells enables a stabilization of the biofilm structure. Moreover, the motility is reduced within bacterial microcolonies due to the inhibition of bacterial surface structures **(Preda and Săndulescu, 2019; Sharma *et al*., 2023)**. Mature biofilms provide a protection for bacteria towards multiple environmental stressors (e.g. desiccation, UV rays, poor nutrients, antibiotics, etc.) **(Yin *et al*., 2019; Muhammad *et al*., 2020)**.

4.6. Biofilm dispersal

Biofilm dispersion represents the last stage in biofilm formation process **(Muhammad *et al.*, 2020)**. After maturation, the biofilm can partially lose its structure which can occur by detachment and/or dispersion of planktonic bacteria **(Guzmán-Soto *et al.*, 2021)**. Once the biofilm reaches a critical mass, the extracellular matrix suppresses the protective layer to allow the biofilm to release the bacterial cells from its surface **(Oopath *et al.*, 2022)**. Thus, these bacteria disseminate in new surfaces in order to colonize them **(Guilhen *et al.*, 2017; Chen *et al.*, 2022)**.

There are two different mechanisms of biofilm dispersal: active (dispersion) and passive (detachment) **(Singh *et al.*, 2021)**. Dispersion is an active release in which biofilm cells actively escape from the biofilm to new colonization surfaces and it induced in response to environmental conditions alteration such as decrease in nutrient and oxygen availability and accumulation of toxic products (e.g. high level of nitric oxide and heavy metals) **(Khatoon *et al.*, 2018; Rumbaugh and Sauer, 2020; Wille and Coenye, 2020)**. As a result, these stress factors active regulatory mechanisms, including the secondary messenger c-di-GMP and stringent response which induce production and release of enzymes to degrade biofilm matrix components (e.g. alginate lyase) inducing cell death thereby leading to the formation of cavities in the biofilm that bacteria can use as a means of escape of the biofilm **(Guzmán-Soto *et al.*, 2021; Singh *et al.*, 2021)**. In contrast, passive detachment implies the release or loss of biofilm portions as consequence of external forces such as shear fluids. Different mechanisms such as abrasion, grazing, erosion and sloughing have been described **(Rumbaugh and Sauer, 2020; Wille and Coenye, 2020; Singh *et al.*, 2021)**.

5. Factors which influence biofilm formation

Biofilm formation is a complex and dynamic process **(Pang *et al.*, 2023)**. However, its development is affected by several factors such us surface charge, surface roughness, hydrophobicity, fluid dynamics and bacterial motility. More details will be given below about these factors.

5.1. Surface charge

Surface charge is one of the important factors that can influence bacterial adhesion to surfaces **(Achinas *et al.*, 2019; Zheng *et al.*, 2021)**. The bacterial cell wall surface has a negative charge due to the presence of carboxyl, amino, and phosphate groups **(Zhao *et al.*, 2023)**. In

addition, several studies showed that bacteria adhere easily to positively charged surfaces **(Kovačević *et al.*, 2016; Guo *et al.*, 2018; Oh *et al.*, 2018; Chen *et al.*, 2019)**. For instance, **Kovačević *et al.* (2016)** demonstrated a greater adhesion of *P. aeruginosa* on a positively-charged poly (allylamine hydrochloride) surface compared to negatively-charged poly (styrenesulfonate) surface. Another study observed that *E. coli* and *S. aureus* adhered more to surfaces with positive charge **(Oh *et al.*, 2018)**. **Chen *et al.* (2019)** showed also an easily adhesion of *E. coli* on cellulose surfaces modified by adsorbed cationic polyelectrolytes.

However, researchers' understanding of the relationship between charge and adhesion remains complicated. It has been reported that some bacteria are able to overcome electrostatic repulsion and can bind to negatively charged surfaces due to their appendages, such as *fimbriae* **(Ueshima *et al.*, 2002)**. Moreover, LPS, a surface component of Gram-negative bacteria helped their attachment to negatively charged surfaces **(Rzhepishevska *et al.*, 2013)**. In contrast, a recent study observed a reduced adhesion of *E. coli*, *P. aeruginosa* and *S. aureus* on positively charged surface **(Shen *et al.*, 2020)**. For that, further research is needed to understand how the surface charge influence on bacterial adhesion.

5.2. Hydrophobicity

The hydrophobicity is an important biophysical parameter that affects the interaction between bacterial cells and surfaces and biofilm formation **(Achinas *et al.*, 2019; Das *et al.*, 2022)**. Studies have shown that bacteria attach and behave differently on hydrophobic and hydrophilic surfaces, making the hydrophobicity of attaching surface and of bacterial cell important for biofilm formation **(Berne *et al.*, 2018; Forson *et al.*, 2020)**.

Bacteria with more hydrophobic cell surfaces prefer to colonize hydrophobic materials and vice versa **(Berne *et al.*, 2018; Danchik and Casadevall, 2021)**. For that, it is relatively easy for hydrophobic bacteria to adhere to medical implants made of hydrophobic materials (silicon, teflon, etc.) **(Krasowska and Sigler, 2014)**. Furthermore, the hydrophobicity affects the strength of adhesion forces and the accumulation velocity during colonization **(Nakanishi *et al.*, 2021)**.

5.3. Surface roughness

The role of surface roughness in bacterial adhesion and biofilm development has been widely studied **(Zheng *et al.*, 2021)**. Surface roughness may be directly related to increased bacterial adhesion **(Achinas *et al.*, 2019)**. Therefore, as the roughness of the surface increases,

bacterial adhesion and biofilm formation also increase **(Yao *et al*., 2020)**. Moreover, a rougher surface can protect bacteria from the shear forces of fluids **(Achinas *et al*., 2019)**.

Some studies have showed a significant correlation between biofilm formation and surface roughness. For example, a study conducted by **James *et al*. (2019)** showed that the adhesion of *S. epidermidis* and *P. aeruginosa* to rougher surfaces was higher compared to the smooth surfaces.

5.4. Hydrodynamics

Hydrodynamics is another important factor that affects bacterial adhesion, biofilm development and its structure and enhances the transport of nutrients and oxygen to the biofilm **(Soares *et al*., 2022)**. This parameter also plays an important role in experiments, because the tests carried out in static conditions do not really imitate what happens in the human body (for example the flow of urine in the urinary tract) **(Gomes *et al*., 2022)**. Many biofilms causing infections in humans grow under dynamic conditions. An example, in the mouth, dental plaques is exposed to the flow of salivary fluid. Another example is the flow of fluids in catheters which affects also biofilm formation **(Zheng *et al*., 2021)**. A study conducted by **Santos *et al*. (2019)** showed that the viability of a biofilm in semi-dynamic conditions, using an artificial oral cavity model, was superior compared to static conditions.

During bacterial adhesion, hydrodynamics controls the rate at which fluids and microorganisms are delivered to surfaces, the duration they remain near the surface, and the shear forces at the surface-fluid interface **(Gomes and Mergulhão, 2021)**. Additionally, the fluid surrounding the biofilm is a source of nutrients and oxygen thereby a higher flow velocity will promote higher nutrients supply to the biofilm **(Gomes and Mergulhão, 2021)**.

In addition to its important role in the adhesion, hydrodynamics influences the structure of the biofilm due to the shear forces. In fact, higher shear forces lead to the formation of denser and stronger biofilms thereby improving their resistance to antimicrobial agents **(Zheng *et al*., 2019; Soares *et al*., 2022)**.

5.5. Bacterial motility

Motility (swimming, swarming and twitching) is an important factor during bacterial adhesion, the first step in biofilm formation **(Ye *et al*., 2022)**. In fact, planktonic bacteria are able to colonize different surfaces using their flagellum, swim towards these surfaces, attach to them and spread to other surfaces thereby contributing to their virulence **(de Sousa *et al*., 2023; Vilas**

Boas *et al*., 2024). Swimming motility is a type of movement in liquid environment that involves the rotation of flagella. Swarming motility is the coordinated movement of bacterial cells with multiple flagella (differentiation into hyper-flagellated cells) across solid surfaces **(Zegadło *et al*., 2023)**. For twitching motility, type IV pili extend, attach to the surface, and then retract to enable the movement **(Palma *et al*., 2022)**.

Mobile bacteria possess a flagellum and a chemotaxis transducer that allows them to search and migrate toward a favorable environment or away from unfavorable environments. However, for non-mobile bacteria, adhesion to surfaces can be achieved through gravitational sedimentation **(Zheng *et al*., 2021)**. In addition, the diffusion coefficient of mobile bacteria is 3 times higher than that of non-motile bacteria, thereby increasing their speed and adhesion rates to surfaces **(Zheng *et al*., 2021)**.

6. Biofilm and antimicrobial resistance

Since their discovery, antibiotics have revolutionized medicine and saved a large number of human lives. Unfortunately in recent years, the failure in infections treatment with antibiotic has continued to increase causing a serious health problem worldwide **(Levin-Reisman *et al*., 2019; de la Fuente-Nunez *et al*., 2023)**. In addition to antibiotic resistance, biofilm formation is considered another important factor contributing to therapeutic failure (**de la Fuente-Nunez *et al*., 2023)** and it has been reported that biofilm is responsible of about 65% of infections **(Haney and Hancock, 2022)**. In fact, bacteria within biofilm are characterized by increased tolerance to antibiotics and their ability to survive and re-grow after treatment is the causes of recalcitrance of biofilm related infections and therapeutic failure. The spread of antibiotic resistance within the tolerant bacterial population exacerbates the situation **(Usui *et al*., 2023)**. Moreover, bacterial cells in biofilm are 100 to 1000 times more resistant compared to their planktonic cells counterparts **(Sabino *et al*., 2022)**.

Two phenomena contribute to biofilm recalcitrance: resistance and tolerance (**Uruèn *et al*., 2020)**. Resistance is the ability of bacteria to grow in the presence of an antibiotic at a concentration that would normally inhibit growth for a long period of time and this phenomenon can be due to an intrinsic resistance, mutations, or genetic elements transfer. Tolerance means the ability of bacteria to survive in the presence of increasing antibiotic concentrations even those over the minimal inhibitory concentration (MIC) without developing resistance. In addition, tolerance is a transitional phenomenon from a "growing" state to a "dormant" state in response to unfavorable conditions such as antibiotic exposure **(Hall and Mah, 2017; Bowler *et***

***al*., 2020; Uruèn *et al*., 2020)**. It is important to know that pathogenic bacteria in a biofilm use both tolerance and resistance mechanisms to resist antimicrobial agents **(Hall and Mah, 2017)**.

Several mechanisms contribute to biofilm resistance to antibiotics including: **(1)** The biofilm matrix that can act as a physical barrier containing different anionic and cationic molecules (proteins, glycoproteins and glycolipids) which can bind to charged antibiotics and give a protection to bacteria **(Lahiri *et al*., 2021; Shree *et al*., 2023)** and also enzymes called secreted β-lactamases which can degrade antibiotics, preventing them from reaching their cellular targets **(Hall and Mah, 2017)**; **(2)** the exchange of resistance genes between cells by horizontal gene transfer, thanks to their close proximity in the biofilm **(Flemming *et al*., 2016; Bowler *et al*., 2020)**; **(4)** the presence of persistent cells that are characterized by slow growth **(Pai *et al*., 2023; Mancuso *et al*., 2024)**; **(5)** the upregulation of efflux pumps which are able to extrude several antibiotics out of the biofilm **(Pai *et al*., 2023)** and **(6)** the *quorum sensing* which regulates the expression of genes involved in antibiotic resistance **(Liu *et al*., 2024)**.

7. Persister cells

Persister cells are a subpopulation of bacterial cells in a dormant state or metabolically inactive without genetic change, exhibit increased tolerance to antibiotics, can stay viable for long periods of time, even in the absence of nutrients or growth factors, and are able to re-grow after antibiotic treatment withdraw leading to new biofilm infection **(Uruèn *et al*., 2020; Hemeg *et al*., 2022; Pai *et al*., 2023)**. In fact, persisters are phenotypic variants identical to wild-type bacteria. Moreover, we should keep in mind that persistence refers to the behavior of a subpopulation towards antibiotics, whereas tolerance concerns the behavior of an entire bacterial population **(Stojowska-Swędrzyńska *et al*., 2023)**. Persister cells are bacterial survival pathway which are formed during environmental stress (nutrient limitation, antibiotics, oxidative stress) and it has been reported that several adaptative mechanisms such as the guanosine pentaphosphate or tetraphosphate ((p)ppGpp)- mediated stringent response, SOS response and toxin-antitoxin systems are involved in persisters formation **(Kim *et al*., 2018; Stojowska-Swędrzyńska *et al*., 2023; Pai *et al*., 2023)**.

Several studies demonstrated that persister cells are strongly involved in chronic infections and their relacitrance in clinical; making the antibiotic treatment ineffective and biofilm eradication impossible **(Yan and Bassler, 2019; Wainwright *et al*., 2021; Stojowska-Swędrzyńska *et al*., 2023; Theis *et al*., 2023)**.

8. Resistance to immune system

In addition to their resistance to antibiotics, microorganisms within biofilm are protected also from the host immune defenses **(Roy *et al*., 2018; Pettygrove *et al*., 2021)**. Indeed, the host immune response is unable to completely eliminate biofilm-related infections, hence the chronicity of these infections **(Arciola *et al*., 2023)**. It has been reported that most studies on immune response have been done against planktonic bacteria. Therefore, little information has been published on the immune response against biofilms **(Moser *et al*., 2017)**. Indeed, they can escape the immune response via different mechanisms which are less well characterized including: the biofilm matrix acts as a physical barrier, allowing bacteria to escape phagocytosis; penetration failure of leukocytes along with their bactericidal products into the biofilm; genetic activation of suppressors which interferes with the immune cell activity; and genetic activation of global regulators responses for bacterial protection **(Leid, 2009; Arciola *et al*., 2018; González *et al*., 2018)**.

Another phenomenon called "frustrated phagocytosis" could also explain the biofilm resistance to phagocytosis. Due to their inability to engulf bacteria within the biofilm, frustrated macrophages and neutrophils will release reactive oxygen species (ROS) which will damage healthy host cells causing very serious harm to patients **(Davis-Fields *et al*., 2019; Arciola *et al*., 2023)**.

9. Cell-to-cell communication (Q*uorum sensing*)

Quorum sensing (*QS*) is a regulating system for bacterial communication between each other within the biofilm which depends on population density, acting via signaling molecules called autoinducers **(Srinivasan *et al*., 2021; Dutt *et al*., 2022)**. It was first described in a bioluminescent marine bacterium called *Vibrio fisheri* **(Li and Zhao, 2020)**. When auto-inducers concentration reaches a certain threshold with bacterial density, the expression or repression of several genes can be started to regulate bacterial behavior **(Verderosa *et al*., 2019; Zhao *et al*., 2020)**.

In fact, the principle of *QS* consists firstly in the production of signal molecules. Once the bacterial density is high, the protein receptor detects these signal molecules in bacterial cytoplasm forming receptor-signaling molecule complexes thereby activating the expression of specific genes **(Raju *et al*., 2022)**. *QS* systems control several bacterial processes such as biofilm formation, dormancy generation, bioluminescence, antimicrobial tolerance, immune escape and virulence factors production **(Li and Zhao, 2020)**. Signal molecules involved in *QS* are different depending on bacteria type: N-acyl-homoserine lactones (AHL) in Gram-negative bacteria, auto-

inducing peptide (AIP) in Gram-positive bacteria and auto-inducing peptide 2 (AI-2) in Gram-positive and Gram-negative bacteria (intra- and inter-species communication) **(Preda and Săndulescu, 2019; Uruèn *et al.*, 2020)**.

The two component system, first described in *Vibrio fisheri*, LuxR-LuxI, is the most studied regulatory system in Gram-negative bacteria. However, other systems are reported, for example, the *QS* in *P. aeruginosa* is mediated by LasI/LasR-RhlI/RhlR system. On the other hand, in Gram-positive bacteria, the *QS* system consists of the interaction between small signal peptides (AIP) and the two-component histidine kinase system. The best known example is the *agr* system in *S. aureus* species **(Li and Zhao, 2020; Prazdnova *et al.*, 2022)**.

It has been reported that *QS*-deficient mutants appear to develop biofilms that are less structured and much less resistant to antimicrobial agents **(Uruèn *et al.*, 2021; de Brito *et al.*, 2022)**. Given the importance of *QS* in biofilm formation and its maturation, the inhibition of this mechanism could be a considerable way to fight against biofilms **(Sharma *et al.*, 2024)**.

Chapter II. Medical device-associated biofilm infections

1. Hospital-acquired infections

Hospital-acquired infections (HAIs) or nosocomial infections (NI) are defined as infections which were neither present nor incubating during the patient's hospitalization and were acquired after 48 h of hospitalization. These infections increase patient morbidity and mortality, prolong their hospital stays, and represent a massive additional financial burden for health structures **(Tassew *et al*., 2020)**. The severity of infection and its incidence is much higher in patients in burn units, intensive care units (ICU), organ transplant receivers, and with newborns due to their immunological status **(Avershina *et al*., 2021)**. In addition to the problems associated with NIs, antibiotic resistance and the emergence of multi drug resistant (MDR)-bacteria are a serious global problem, due to the uncontrolled administration of drugs **(Yang *et al*., 2021)**.

These HAIs are often the result of the use of invasive procedures such as the location of temporary indwelling devices (Venous and urinary catheters, endotracheal tubes, and wound drains) or are associated to the placement of cardiovascular or orthopedic implants during a surgical intervention **(Figure 2) (Dadi *et al*., 2021)**. They include a wide range of infections such as catheter- associated bloodstream infections (CA-BSIs), catheter associated-urinary tract infections (CA-UTIs), and ventilator associated pneumonia (VAP) **(Percival *et al*., 2015)**. These infections are generally designed as "Medical device-associated biofilm infections" **(Zhang *et al*., 2020)**.

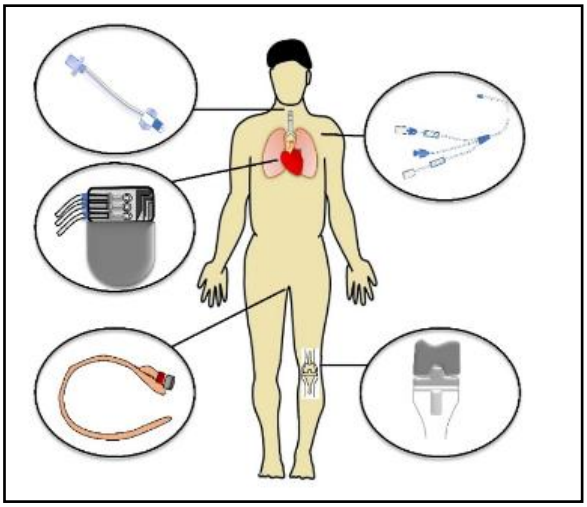


Figure 2. The most common locations of medical devices **(Dadi *et al*., 2021)**

Several Gram-negative (*E. coli, K. pneumoniae, Proteus mirabilis, P. aeruginosa,* and *A. baumannii*) and Gram-positive (*S. aureus, S. epidermidis,* and *En. faecalis*) bacteria are involved

in the onset of NIs **(Vergalito *et al*., 2019; Gmiter and Kaca, 2022)**. Their ability to form a biofilm makes the treatment of these infections more complicated **(Khatoon *et al*., 2018)**. In fact, it is well known that biofilms have a significant impact in medicine through the development of HAIs **(Olivares *et al*., 2020)** and it is estimated that bacterial biofilms are involved in 65% of NIs and in more than 80% of chronic infections **(Jamal *et al*., 2018)**. Treatment of these infections requires administration of high dose antibiotics and/or replacement of the device, which are both ineffective due to the antibiotic resistant strains and the high risk of re-infection on the new device **(Khatoon *et al*., 2018)**.

2. Catheter- associated bloodstream infections (CA-BSIs)

2.1. Intravenous catheterization

Intravenous catheterization is an invasive method commonly used in healthcare settings **(Kaur *et al*., 2019)**. Despite the practical skills of the medical staff, complications cannot be excluded **(Bodenham, 2017)**. This practice is frequently used in several situations such as drugs, fluids and blood derivatives administration, collection of blood and during hemodialysis **(Jayaweera and Sivakumar, 2020)**.

The peripheral intravenous catheter (PVC) is a hollow, short-tube, plastic catheter that is connected to a larger hub that remains over the skin. This device is usually inserted through the skin into peripheral veins such as that in the forearm, hand, leg and foot **(Bertoglio *et al*., 2017; Beecham and Tackling, 2021)**. Most catheters are made of silicone, polyurethane or polytetrafluoroethylene (Teflon) **(Guanche-Sicilia *et al*., 2021)**. Once inserted, PVC can remain in place for several days if no problem is noticed to continued treatment of the patient without the need to repeatedly insertion of a new needle **(Bertoglio *et al*., 2017; Beecham and Tackling, 2021)**.

The diameter of the catheters is calculated using a gauge unit. Their inner diameter varies between 14G and 24G; the higher the gauge number, the narrower the catheter. Catheters with sizes between 20 and 24G are most highly recommended. Nevertheless, the appropriate diameter and length of the catheter are chosen according to the patient's age and veins. It should be noted that the catheter length is also important in obese patients with deep veins **(Bertoglio *et al*., 2017; Beecham and Tackling, 2021)**. PVCs are common devices in healthcare units and are used for a short time catheterization **(Drugeon *et al*., 2023; Zingg *et al*., 2023)**.

A central venous catheter (CVC) is a device that is inserted peripherally within a large central vein (internal jugular, subclavian, or femoral vein) and advanced until the terminal lumen

resides in the inferior *vena cava*, the superior *vena cava*, or the right atrium **(Kolikof *et al*., 2021)**. These types of catheters are designed for medium/long period of time **(Casimero *et al*., 2020)** and are used for the following indications: nutritional fluids administration, chemotherapy, hemodialysis, medications infusion, central venous pressure monitoring, pulmonary artery catheterization, emergency venous access in patients who cannot use peripheral catheters **(Casimero *et al*., 2020; Tse and Schick, 2021)**.

2.2. Physiopathology of CA-BSIs

There are several pathways involved in catheter contamination: colonization of the surface of catheter tip by skin microorganisms originating from healthcare worker's hands and/or contaminated disinfectant, which migrate to the insertion site in the skin pathway of the catheter and along the catheter surface (extraluminal) and this is the most common route of infection for short-term catheters (inserted for ≤14 days), colonization of the inner surface of the catheter by contaminated infusion product and catheter hub (intraluminal) and finally, by hematogenous seeding (rare route) **(Gahlot *et al*., 2014; Rupp and Karnatak, 2018)**.

In contact with blood, microorganisms interact with fibrin to produce an adherent biofilm which will promote bacterial colonization and their spread **(Figure 3) (Rupp and Karnatak, 2018)**. Colonization and biofilm formation on the catheter surface occurs 24 h after device insertion **(Soi *et al*., 2016)**. These pioneer pathogens allow the attachment of other bacteria by providing more diverse adhesion sites. After multiplication, the bacteria produce an extracellular matrix which maintains the biofilm, leading to irreversible adhesion to the surface of the catheter **(Gominet *et al*., 2017)**. Once the biofilm is mature, bacteria can disperse, cause CA-BSIs and colonize other sites in the body **(Lebeaux *et al*., 2014)**.

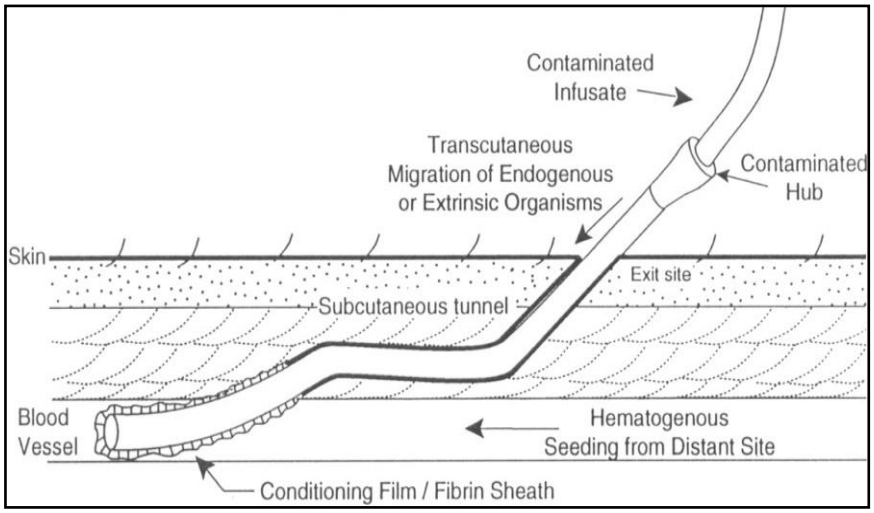


Figure 3. Source of bacterial contamination of intravenous catheters **(Rupp and Karnatak, 2018)**

The formation of fibrin sheaths is also observed. Once the catheter is inserted, the fibrinogen, albumin, lipoproteins and coagulation factors, released due to the lesion of the blood vessels, begin to deposit on the surface of the catheter within 24 h forming a fibrin sheath which will cover the surface of the catheter within days or even weeks. This fibrin sheath is responsible for the catheter dysfunction, which mostly happens about three months after the catheter placement **(Neoh *et al*., 2017; Bandyopadhyay *et al*., 2020)**.

2.3. Diagnosis of CA-BSIs

Two methods are used for catheter colonization evaluation, the semi quantitative method of Maki and the quantitative method of Brun-Buisson. According to them, catheter colonization is defined as a semi-quantitative culture ≥ 15 colony-forming units (CFU) or a quantitative culture ≥ 10^3 CFU/mL respectively **(Timsit *et al*., 2020)**. However, the diagnosis of a CA-BSI is based on a combination of a catheter culture ≥ 10^3 CFU/mL or ≥ 15 CFU and local symptoms (purulent discharge or abscess at the catheter insertion site) and/or systemic symptoms (fever or organ dysfunction), with complete or partial disappearance of symptoms of infection within 48 h after catheter removal **(Timsit *et al*., 2020)**. CA-BSI means that patients with an intravascular catheter have at least:

- A positive blood culture taken from a peripheral vein, presence of signs of infections and a positive semi-quantitative (≥15 CFU) or quantitative (≥10^3 CFU/mL) culture from the tip of the catheter that identifies the same microorganism, with no other evident origin for the blood infection than the catheter **(Gahlot *et al*., 2014)**;

- Positive paired blood cultures for the same microorganism, obtained simultaneously from the catheter and the peripheral vein blood, showing that the colony count in the catheter's lumen blood is three times greater than that in the blood collected from the vein **(Rupp and Karnatak, 2018)**;

- A positive blood culture from the venous catheter, obtained 2 h prior to that from the blood drawn from the peripheral vein. This method is used for diagnosis of CA-BSIs without removing the catheter **(Ruiz-Giardin *et al*., 2019; Orihuela-Martín *et al*., 2020)**.

Upon confirming a diagnosis of a blood infection related to the catheter, the venous catheter must be removed and a new one placed. However, for the patients whose catheter is essential for their survival, development of anti-biofilm strategies is primordial **(Wolcott *et al*., 2021)**.

3. Catheter-associated urinary tract infections (CA-UTIs)

3.1. Bladder catheterization

In situations where normal bladder voiding is impeded due to surgery, illness, or injury, the use of a urinary catheter (URC) may become essential **(Figure 4A) (Murphy, 2018)**. A URC consists of a long, flexible tube that is inserted into the urethra **(Donlan, 2001b; Costese *et al*., 2018)**. These devices primarily facilitate urinary drainage but also help managing urinary incontinence, irrigate the bladder, contribute in urological surgeries or other operative procedures, assist in the recovery of perineal wounds in individuals with incontinence, enhance comfort in end-of-life care when necessary **(Meddings *et al*., 2014)**. It is estimated that 15–25% of hospitalized patients and 75% of those at critical care require this intervention **(Yang *et al*., 2019b)**.

The Foley catheter is the most commonly used URC worldwide **(Kim and Chung, 2023)**. This device contains a tube with two channels: the larger channel facilitates urine to exit the bladder, while the smaller channel is responsible for the inflation of the balloon located just below the catheter's end at the bottom of the bladder. When filled with water, this balloon holds the catheter in place, preventing it from falling out until removal. Additionally, the catheter is connected to an extended tube that directs urine into a drainage bag for collection **(Cortese *et al*., 2018) (Figure 4B)**.

Furthermore, the drainage bag needs to be emptied at least every 4 to 6 h or when it reaches half full **(Bradley *et al*., 2018)**. Urine can be drained outside the bladder by inserting a

Foley catheter through the urethra (transurethral catheterization) which is the most frequently used technique or by creating an entry point in the lower abdomen, above the pubic bone (suprapubic catheterization) **(Newman *et al.*, 2017)**. The female urethra is shorter (long ~40 mm), muscular, and straight, in contrast to the male urethra which is longer (long ~160 mm), more sensitive, and curved, making catheter insertion potentially painful and prone to complications **(Feneley *et al.*, 2015)**. A URC can be inserted for a temporary period or for a long duration based on the patient's mobility and health condition **(Costese *et al.*, 2018)**. Generally, URCs are classified as short-term if used for less than one month and long-term if utilized for one month or more **(Nicolle, 2014; Lachance *et al.*, 2019)**.

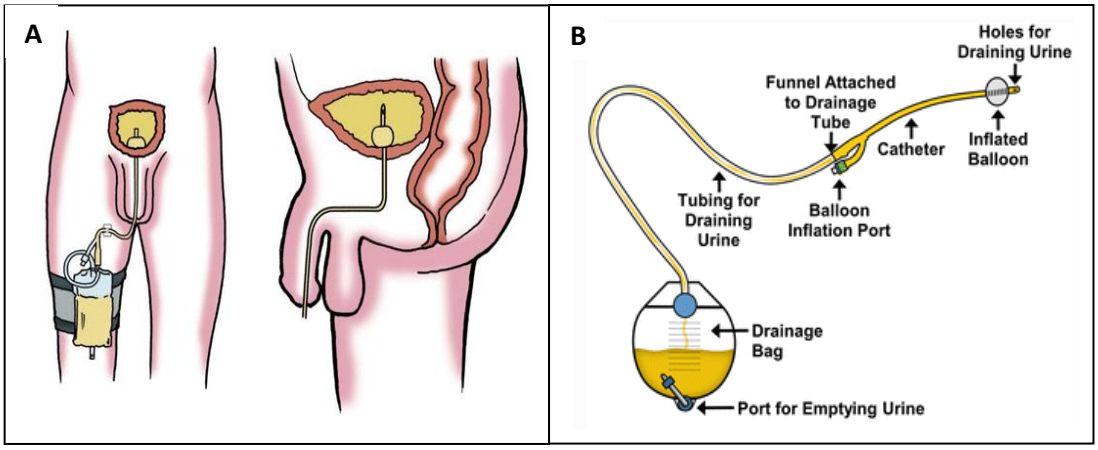


Figure 4. (A) Man with indwelling urinary catheter **(Murphy, 2018); (B)** Foley catheter system **(Newman *et al.*, 2017)**.

3.2. Choice of a urinary catheter

Many criteria should be considered when selecting a URC including: **(1) size**: The diameter of a URC is expressed in French (F) unit in which 1F equals to 1/3 mm **(Agwu *et al.*, 2022)**. Catheters from 14 to 18F are appropriate for men, 12 to 14F for women, and 6 to 10F for child. Moreover, the smallest diameter should be choosing for a good urinary drainage and to ovoid urethral trauma and stenosis **(Gilbert *et al.*, 2018b; Newman, 2021)**. In addition, URC sizes are listed by color. For example, black for 10F, white for 12F, green for 14F and orange for 16F **(Newman, 2021)**; **(2) length**: A standard male catheter measures 40–45 cm in length; a female catheter is between 20–26 cm long, whereas a pediatric catheter ranges from 30–31 cm in length **(Akanmode *et al.*, 2020)**; **(3) type of material**: URCs are made from different polymers, including latex (mainly), polyvinylchloride (PVC) and silicone (**Costese *et al.*, 2018)**. Latex is recognized for its adaptability and affordability. Nonetheless, it can cause allergic responses and is susceptible to encrustation and infections **(Newman, 2021)**. The development of coated latex

catheters with silicone (e.g. silicone elastomer), polytetrafluoroethylene (Teflon), hydrogels, poly(ethylene glycol) could overcome its drawbacks (prevention of bacterial colonization and encrustation, higher biocompatibility, reduce urethral irritation) **(Markowitz *et al.*, 2018; Andersen and Flores-Mireles, 2020)**. Additionally, the latex catheters are advisable for short term catheterization (<7days) whereas URCs made from silicone or coated catheters are advisable for long term catheterization (~12 weeks) **(Reid *et al.*, 2021)**.

3.3. Diagnosis of CA-UTIs

To categorize an infection as a CA-UTI, a patient must have the following criteria: (i) a URC inserted for more than 48 h; (ii) at least one clinical manifestation (e.g. fever, pain, suprapubic tenderness, frequent or urgent urination or painful urination; and (iii) a positive urine culture with $\geq 10^5$ CFU/mL of bacteria **(Letica-Kriegel *et al.*, 2019)**. Nevertheless, there is considerable debate regarding the CFU/mL threshold in samples taken from a URC, with multiple authorities arguing that a count of $\geq 10^3$ CFU/mL signifies a true CA-UTI **(Maki and Tambyah, 2001; Parida and Mishra, 2013; Crader and Leslie, 2021)**.

3.4. Physiopathology of CA-UTIs

Transurethral ascension of microorganisms is the most common mechanism for the development of UTIs, which explains the increased risk of infection after catheterization **(Köves *et al.*, 2017)**. Bacteria can colonize URCs either by endoluminal route which involves exogenous flora originating from colonization of the collecting sac or from a breach of the closed system during manipulations of the URC, or by exoluminal route which involves the endogenous flora of the urinary meatus and occurs early during catheter placement or later following colonization of the urinary meatus by the intestinal flora **(Aumeran *et al.*, 2021) (Figure 5A)**.

After insertion of the catheter, the bacteria overcome firstly the electrostatic repulsion observed between bacterial cell and catheter surface to allow intimate interactions to occur, then adhere to a conditioning film of urine components and host proteins, such as Tamm-Horsfall protein, magnesium and calcium ions, which form along the catheter surface. It also reported that the URC elicits an inflammatory response resulting in the release of the host protein fibrinogen into the lumen of the bladder which will cover the surface of the catheter **(Xu *et al.*, 2017)**. Pathogens such as *S. aureus* and *En. faecalis* possess adhesins such as the fibronectin-binding protein A (FnBPA) and the endocarditis and biofilm-associated pilus (EbpA), respectively, which bind to the host fibrinogen in order to disrupt blood clotting, initiate biofilm formation as well as immune evasion **(Flores-Mireles *et al.*, 2016; Pickering *et al.*, 2019)**.

Once irreversibly fixed to the surface of the catheter via adhesins and pili, bacteria begin to change their phenotype, producing exopolysaccharides (EPS) which protect them and form a biofilm **(Jacobsen *et al.*, 2008; Flores-Mireles *et al.*, 2015) (Figure 5B)**. The presence of biofilms promotes the appearance of epithelial lesions due to the proteases and bacterial toxins produced. The uropathogenic bacteria can then ascend to the kidneys, attaching again to the renal epithelium, causing kidney infections. Left untreated, these infections can progress to bacteremia by crossing the tubular epithelial cell barrier into the bloodstream **(Flores-Mireles *et al.*, 2015)**.

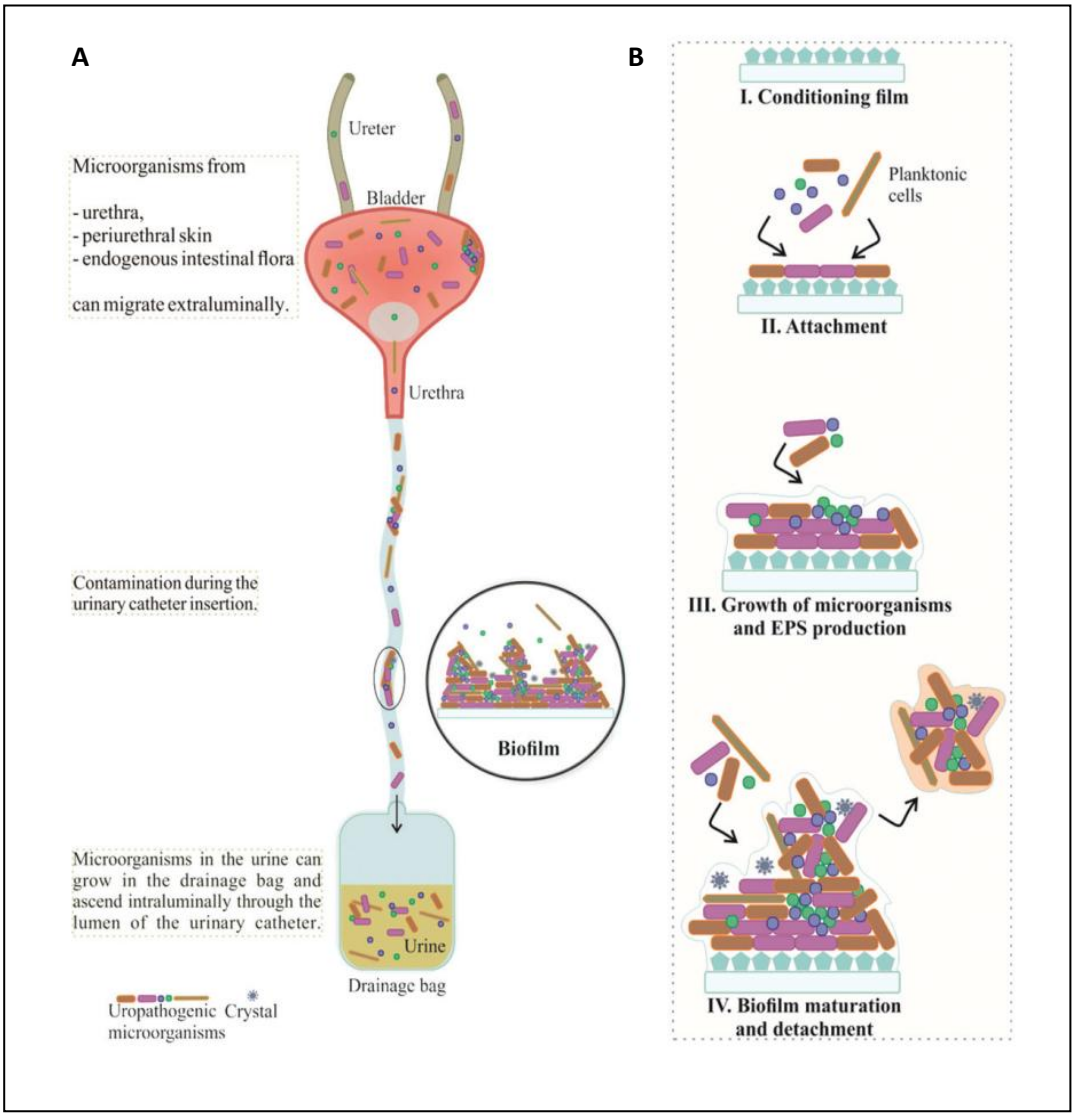


Figure 5. Physiopathology of catheter-associated urinary tract infections: (A) Routes of entry of uropathogens to catheterized urinary tract, (B) Biofilm formation steps on urinary catheter **(Azevedo *et al.*, 2017)**.

Chapter III. Most common pathogenic bacteria involved in medical device-associated biofilm infections

Several reports showed that Gram-positive and Gram-negative bacteria cause up to 70% of NIs in hospitalized patients **(Mishra *et al.*, 2024)**. The most reported causative pathogenic bacteria of these infections are *E. coli*, *K. pneumoniae*, *A. baumannii*, *S. aureus*, *S. epidermidis* and *En. faecalis* **(Dadi *et al.*, 2021; Zhao *et al.*, 2023)**. In this chapter, we will focuse on these species. Moreover, more details can be found in the review "Medical Device-Associated Biofilm Infections and Multidrug-Resistant Pathogens" **(Bouhrour *et al.* 2024)**.

1. *Escherichia coli*

E. coli is responsible for a wide variety of community infections and HAIs such as UTIs and BSIs with increasing antimicrobial resistance rates **(Cergole-Novella *et al.*, 2015; Luden *et al.*, 2021)**. Indeed, These strains have become more resistant to antibiotics with the increasing prevalence of extended-spectrum β-lactamases (ESBLs) **(Cantas *et al.*, 2016; D'Incau *et al.*, 2023)**. In addition to the global emergence of resistance to carbapenems, the significant increase in the prevalence of quinolones-resistant *E. coli* strains has also been reported in several countries limiting the treatment choice for these bacterial infections, thereby constituting a real problem for public health **(Benduki *et al.*, 2021; D'Incau *et al.*, 2023)**. Furthermore, One of the *E. coli* pathotypes, Extraintestinal pathogenic *E. coli* (ExPEC), occupies a specific place in the human gut microbiota and can lead to extraintestinal infections such urinary tract infections (including CAUTIs), meningitis, septicemia, etc **(Sora *et al.*, 2021)**. Uropathogenic *E. coli* (UPEC) which is a sub-category of Extra-intestinal E.coli (ExPEC), is the bacterium mostly involved in 80 to 90% of UTI cases **(Sora *et al.*, 2021; Terlizzi *et al.*, 2017; Valiati *et al.*, 2020)** and in 40% of hospital acquired UTIs **(Kumar *et al.*, 2022)**.

E. coli is one of the most implicated bacteria in biofilm-related infections, particularly in CA-UTIs **(Weiner *et al.*, 2016; Musinguzi *et al.*, 2019; Ballén *et al.*, 2022; Alshammari *et al.*, 2023; Nye *et al.*, 2024)** and CVC-BSIs **(Ripa *et al.*, 2018; Tsuboi *et al.*, 2018; Surapat *et al.*, 2020; Lendak *et al.*, 2021)**. In fact, biofilm formation is the major cause of these infections in catheterized patients, making them hard to eradicate **(Sharma *et al.*, 2015; Zou *et al.*, 2023)**. Indeed, their ability to form a biofilm is associated with the persistence and chronicity of inflammations leading to complicated and/or recurrent infections **(Delcaru *et al.*, 2016; Katongole *et al.*, 2020)**.

E. coli strains have an arsenal of virulence factors which contribute to adhesion, colonization, and persistence allowing the different defense mechanisms of the host to be

overcome **(Dadi *et al.*, 2020)**. Among them, we distinguish adhesins, *fimbriae*, toxins, siderophores, etc. **Table 1 (Appendix I)** summarizes the virulence factors implicated in *E. coli* pathogenesis and especially adhesion and biofilm formation. The role of virulence genes of *E. coli* in its adhesion to catheter surfaces has been reported in several studies **(Reisner *et al.*, 2014; Sharma *et al.*, 2015; Hojati *et al.*, 2015; Inegol Paykoc and Turkyilmaz, 2018; Katongole *et al.*, 2020; Sarshar *et al.*, 2020; Carere-Sigl *et al.*, 2021; Ballén *et al.*, 2022a; Ramírez Castillo *et al.*, 2023; Zou *et al.*, 2023)**. For example, **Reisner *et al.* (2014)** reported that 73% of *E. coli* strains isolated from catheterized patients expressed type 1 *fimbriae*. In a recent study of **Zou *et al.* (2023)**, they showed that biofilm associated genes such as iron transport systems (ferric citrate) and antigen 43 may be involved in the pathogenic CA-UTI strains.

2. *Klebsiella pneumoniae*

K. pneumoniae is considered to be one of the most important opportunistic pathogens responsible for NIs including sepsis, soft tissue infections, pneumonia **(Gorrie *et al.*, 2022)**, and UTIs, which are the most common worldwide **(Ballén *et al.*, 2021)**. *K. pneumoniae* is implicated in 6–17% of opportunistic UTI cases mainly linked to bacterial adhesion in the inner and outer surfaces of the URC **(Mączyńska *et al.*, 2021; Kaye *et al.*, 2024)**. Moreover, it is the second pathogen involved in BSIs, after *E. coli* **(Hafiz *et al.*, 2023)**. The prevalence of NIs due to *K. pneumoniae* has been reported to be approximately 10% worldwide **(Ibrahim, 2023)**.

Several studies revealed the implication of *K. pneumoniae* in HAIs, especially in CA-BSIs **(Barbadoro *et al.*, 2015; Ripa *et al.*, 2018; Yoshida *et al.*, 2019; Pitiriga *et al.*, 2020; Oleksy-Wawrzyniak *et al.*, 2021; Vidal-Cortés *et al.*, 2022; Shen *et al.*, 2022; Hafiz *et al.*, 2023; Zhu *et al.*, 2023)**. Since this bacterium is commensal in humans, gastrointestinal colonization represents the major source of transmission and the development of infections towards other sites **(Chang *et al.*, 2021)**.The global antibiotic resistance rate of *K. pneumoniae* is approximately 70% with mortality rates ranging from 40% to 70% **(Li *et al.*, 2022)** and the emergence and spread of MDR-strains of *K. pneumoniae* have become a real global problem **(Wang *et al.*, 2020a)**. In fact, this bacterium is able to acquire resistance genes such as ESBLs or carbapenemases (resistance to cephalosporins or third-generation carbapenems), limiting treatment options for infections **(Gorrie *et al.*, 2022)**, causing serious or even fatal infections, while also increasing the length of hospitalization and the costs of processing **(Snitkin *et al.*, 2017)**. Moreover, carbapenem-resistant *K. pneumoniae* are the major cause of BSIs with high rates of mortality and morbidity in the world **(Zhu *et al.*, 2023)**. Another type of *K. pneumoniae* strains called “hypervirulent”, able to express acquired virulence factors, has also emerged causing serious community-acquired infections **(Wyres *et al.*, 2020)**.

Many *K. pneumoniae* isolates are able to form biofilms, resulting in increased impermeability to antibiotics causing treatment failure **(Shadkam *et al*., 2021)**. For this, the World Health Organization (WHO) classifies this bacterium among the high priority species and encourages the development of new antimicrobial molecules in order to counter its antibiotic resistance **(Ballén *et al*., 2021)**. *K. pneumoniae* has several virulence factors, including types 1 and 3 *fimbriae*, capsule polysaccharides, LPS and *QS* **(Vuotto *et al*., 2017)** used to escape host immune defenses, biofilm formation, and to persist during infection **(Nirwati *et al*., 2019)**.The adhesins of *K. pneumoniae* allow the establishment of strong biofilms **(Stahlhut *et al*., 2012; Alcántar-Curiel *et al*., 2013; Murphy *et al*., 2013; Wasfi *et al*., 2016; Ferreira *et al*., 2019; Devanga Ragupathi *et al*., 2020; Ochońska *et al*., 2021; Makhrmash *et al*., 2022; Li and Ni, 2023)**. An *in vitro* study reported the improvement of biofilm formation on URCs by the presence of types 1 and 3 *fimbriae* in a bladder model **(Stahlhut *et al*., 2012)**. In a recent study, the results showed that 87.5%, 46.4%, and 53.6% of strains harbored *fim*H, *mrk*A, and *mrk*D fimbrial genes, respectively **(Makhrmash *et al*., 2022)**. Other genes have been reported to be involved in biofilm formation **(El Fertas-Aissani *et al*., 2013; Alcántar-Curiel *et al*., 2013; Chen *et al*., 2014; Devanga Ragupathi *et al*., 2020; Vargas *et al*., 2019; Mirzaie *et al*., 2021; Li and Ni, 2023)**. The main virulence factors of *K. pneumoniae* are summarized in **Table 1 (Appendix I)**.

3. *Acinetobacter baumannii*

A. baumannii is a nosocomial pathogen that is responsible for a large number of infections in humans, including endocarditis, UTIs, meningitis, pneumonia (in mechanically ventilated patients), and sepsis **(Lee *et al*., 2017)**.The incidence rate of *A. baumannii* infections is estimated to be approximately one million cases per year in the world, with high mortality rates, especially in critically ill patients **(Cavallo *et al*., 2023)**. The high prevalence of MDR- *A. baumannii* has become a serious situation in a healthcare setting. One of the major factors responsible for the chronicity and persistence of infections and resistance to antibiotics of *A. baumannii* is its ability to colonize and form a biofilm on biotic and abiotic surfaces (e.g. vascular catheters, cerebrospinal fluid shunts, or Foleys catheter) **(Yang *et al*., 2019a; Zeighami *et al*., 2019)**. In addition to MDR-*A. baumannii*, extremely-drug resistant (XDR) and pan-drug resistant (PDR) isolates have also been reported worldwide **(Cavallo *et al*., 2023)**.

Most *A. baumannii* infections occur in ICU-patients and account for up to 20% of ICU-infections worldwide **(Lee *et al*., 2017; Ceparano *et al*., 2022)**, with an increase in mortality rates (30% to 75%) **(Elbehiry *et al*., 2023)**. Moreover, its ability to acquire resistance to antibiotics and persistence in the environment are the main factors contributing to its survival in

the hospital environment **(Whiteway *et al*., 2022)**. CA-BSIs associated with MDR-*A. baumannii* biofilms have been widely reported **(Pour *et al*., 2011; Lin and Lan, 2014; Greene *et al*., 2016; Blot *et al*., 2020; Kim *et al*., 2020b; Di Domenico *et al*., 2021; Assad *et al*., 2021; Opoku-Asare *et al*., 2023)**. *A. baumannii* possess several adhesins **(Table 1, appendix I)**, which contribute to biofilm formation and bacterial colonization on medical devices **(Cavallo *et al*., 2023)**.

Several reports have shown the presence of *omp*A (detection from 81 to 100%), *csu*E (detection from 80 to 100%), and *bap* (detection from 43 to 91.4%) in clinical *A. baumannii* strains producing strong biofilms **(Azizi *et al*., 2016; Thummeepak *et al*., 2016; Ghasemi *et al*., 2018; Zeighami *et al*., 2019; Khoshnood *et al*., 2020; Li *et al*., 2021c)**. In a recent study, **Kasperski *et al.* (2023)** reported that 72% of strong biofilm producer *A. baumannii* strains harbored four genes associated with biofilm formation (*bap*, *bfm*S, *csu*E, and *omp*A), showing their implication in bacterial adhesion on surfaces.

4. *Staphylococcus aureus*

S. aureus is a clinical pathogen that causes infections in both humans and animals, ranging from mild infections to severe invasive life-threatening infections **(Reddy *et al*., 2017)**. This organism is also the main agent involved in NIs due to its incredible ability to adhere to the surface of medical devices and form a biofilm, which often leads to chronic infections such as osteomyelitis, endocarditis, catheter-associated infections, prostheses-associated infections, and other medical device-associated infections **(Kong *et al*., 2018; Lee *et al*., 2018; Graf *et al*., 2019)**.

S. aureus is one of the major pathogens responsible for nosocomial blood infections with an incidence estimated between 10–30 cases/100,000 persons/year and associated with mortality rates ranging between 15 and 40% **(Kimming *et al*., 2021)**. In addition to this, the global emergence of methicillin-resistant *S. aureus* (MRSA) is a major public health concern given its high virulence and therefore, treatment failure is unavoidable **(Selvaraj *et al*., 2019)**, increasing mortality and morbidity in patients **(Kong *et al*., 2018)**.

It is important to note that the *mec*A gene is the genetic determinant of methicillin resistance in MRSA, which is located on the mobile genetic element called staphylococcal cassette chromosome mec (*SCCmec*) **(Romero *et al*., 2021)**. It has a remarkable arsenal of virulence factors **(Table 2, appendix I)** including toxins, proteases and nucleases, but also many proteins anchored in the cell wall which are important factors allowing it to adhere to tissues, to surfaces, to form biofilm, and to escape the host's immune defense **(Foster *et al*., 2014; Pietrocola *et al*., 2017)**. It has been reported that the mortality rates associated with MRSA

bacteremia are higher than those associated with methicillin-sensitive *S. aureus* (MSSA) bacteremia **(Ishikawa *et al*., 2021)**.

CA-BSIs due to *S. aureus* are considered to be the most fearful of infections. Indeed, **Mandolfo *et al.* (2020)** identified 113 CA-BSIs caused by MRSA (47.5%) and MSSA (52.5%) in hemodialysis patients. In the study of **Bonnal *et al.* (2015)**, 56% of *S. aureus* in PVC-BSIs and 34% of *S. aureus* in CVC-BSIs were identified. Recently, **Pinto *et al.* (2022)** identified *S. aureus* as the main causative agent of CA-BSIs with a rate of 24.1%. Other studies **(Cuervo *et al*., 2015; Kaur *et al*., 2015 ; Hogan *et al*., 2016; Ripa *et al*., 2018; Yoshida *et al*., 2019; Ishikawa and Furukawa, 2021; Bolormaa *et al*., 2023; Weldetensae *et al*., 2023; Mazuel *et al*., 2024**) also reported implication of *S. aureus* in CA-BSIs.

S. aureus, especially MRSA, also constitutes a serious problematic pathogen in CA-UTIs **(Walker *et al*., 2017)**. In fact, *S. aureus* can colonize the urinary tract via urinary catheterization causing ascending UTIs. The presence of MRSA complicates the situation, extending the length of hospital stay **(Alshomrani *et al*., 2023)**. This pathogen causes approximately 0.2%–4% of UTIs and is more often found in patients in long-term care and with long-term catheters **(Mason *et al*., 2023)**. Several studies have shown the implication of *S. aureus* in CA-UTIs **(Walker *et al*., 2017; Maharjan *et al*., 2018; Bagali and Mantur, 2021)**.

Various reports showed the implication of virulence genes in *S. aureus* healthcare infections including CRIs. The major factor in staphylococcal biofilm formation is the polysaccharide intercellular adhesin (PIA) or poly-N-acetyl β-1-6 glucosamine (PNAG), encoded by the *ica*ADBC operon **(Seng *et al*., 2017; Foster *et al*., 2020; François *et al*., 2023)**. This polysaccharide plays an important role in colonization, biofilm formation and biofilm-related infections, antimicrobial resistance, immune evasion, and phagocytosis escape **(Idrees *et al*., 2021)**. *S. aureus* contains also a range of proteins categorised as "microbial surface component recognising adhesive matrix molecules (MSCRAMM)" **(Magro *et al*., 2017; Idrees *et al*., 2021)**, such as fibronectin-binding proteins (FnBPA and FnBPB), clumping factors (ClfA and ClfB), collagen adhesin (Can), elastin binding protein (EbpS), fibrinogen binding protein (Fib), laminin-binding protein (Eno), and serine aspartate repeat proteins C, D, and E (*Sdr*C, *Sdr*D, *Sdr*E) **(Kot *et al*., 2017; Uribe-García *et al*., 2021)**, which have been implicated in binding host matrix components (fibronectin, fibrinogen, collagen) to initiate cell attachment and/or biofilm formation **(Moormeier *et al*., 2017)**. The association between the MSCRAMMs adhesins ClfA/B, FnbA/B, and Cna with bacteremia and catheter-related bacteremia has also been reported **(Giulieri *et al*., 2016; San-Juan *et al*., 2017)**. Similarly, **Walker *et al.* (2017)**

reported that the clumping factor ClfB, interacting with fibrinogen, facilitates colonization of the URC and bladder leading to infection in mice and humans in a CA-UTI model.

5. *Staphylococcus epidermidis*

S. epidermidis is one of the most ubiquitous opportunistic pathogens **(Spoto *et al.*, 2022)** that causes serious infections in immunocompromised patients, especially those associated with the presence of invasive medical devices (e.g. Venous catheter and artificial heart valves) **(Oliveira *et al.*, 2021)**. However, this bacterium rarely causes CA-UTIs and rarely pyelonephritis (without an indwelling urinary device) **(Yogo *et al.*, 2022)**. It is estimated that 30% of CA-BSIs are due to *S. epidermidis* strains **(Pouget *et al.*, 2023)**. A recent report revealed that this species was the most causative agent in CA-BSIs (13.3%) in the emergency department **(Ahn *et al.*, 2023)**. Other prevalence rates have been also reported: 28% **(Strasheim *et al.*, 2015)**, 31% **(Ehlers *et al.*, 2018)**, 7.7% **(Yoshida *et al.*, 2019)**, 18.1% **(de la Cruz-Hernández *et al.*, 2020)**, 31.37% **(Kochanowicz *et al.*, 2022)**, 12.33% **(Pouget *et al.*, 2023)** and 8.3% **(Zaragoza Rodríguez *et al.*, 2023)**. The European Center for Disease Prevention and Control reported that *S. epidermidis* caused 23.6% of CA-BSI cases in ICUs **(Pinto *et al.*, 2022)**. It has also been reported that children are very susceptible to infection associated-methicillin-resistant *S. epidermidis* strains in perinatal units **(Cabrera-Contreras *et al.*, 2019)**.

In addition, in recent years, the situation has become complicated with the capacity of *S. epidermidis* strains to form biofilms on medical devices and their increased resistance to antibiotics, mainly methicillin resistance, leading to significantly high mortality and morbidity rates and medical costs **(Wang *et al.*, 2020b; Xu *et al.*, 2020)**. It has been reported that more than 70% of *S. epidermidis* strains are resistant to methicillin, which is encoded by *mecA* gene, making the treatment of infections ineffective but above all that this resistance to methicillin can be quickly disseminated to other Gram-positive strains via horizontal gene transfer **(Siciliano *et al.*, 2023)**. *S. epidermidis* strains produce a variety of virulence factors contributing to their pathogenicity **(Table 2, appendix I)**. However, unlike *S. aureus*, *S. epidermidis* isolates are weakly virulent, do not produce aggressive toxins, and are commonly non-hemolytic **(Joubert *et al.*, 2022)**. The main virulence factor is its ability to adhere to the surface of medical devices and form a biofilm **(Ardon *et al.*, 2019; Skovdal *et al.*, 2022)**. During accumulation, *S. epidermidis* produces a major component of the biofilm matrix which is PIA **(Oliveira *et al.*, 2021)**. As for *S. aureus*, PIA, encoded by *ica* opeon, is an essential factor in *S. epidermidis* biofilms allowing their adhesion to surfaces **(Ahmad *et al.*, 2022)**. **Cherifi *et al.* (2013)** found that the *ica* operon was significantly more present in CA-BSI isolates than in commensal isolates. However, **François *et al.* (2023)** reviewed that the expression of *ica* operon is not essential in the

colonization of a surface, and the presence of other virulence genes such as *atl*E, *fbe*, and *embp* are involved in catheter-related infections linked to *S. epidermidis* biofilms **(Pouget *et al*., 2023)**.

6. *Enterococcus* spp.

Enterococci are Gram-positive, non-motile, lactic acid-producing bacteria widely found in the gut microbiota of humans and animals **(Garcia-Solache *et al*., 2019)**. Enterococci are facultative anaerobes that tolerate a variety of environmental conditions such as extreme pH, salinity, and a wide temperature range (10 to>45°C) **(Ramos and Morales, 2019)**. Enterococci are tenacious microorganisms characterized by increased tolerance to desiccation and starvation, making them resistant to environmental stresses **(Cui *et al*., 2020)**.

The emergence of *Enterococcus* strains as HAIs agents **(Top *et al*., 2018)** can be explained by the following: (i) these bacteria are intrinsically resistant to several classes of antibiotics (cephalosporins, macrolides, clindamycin, and trimethoprim–sulfamethoxazole) and they acquired resistance to ampicillin, ciprofloxacin, high-level aminoglycosides, and vancomycin **(Arshadi *et al*., 2018)**, causing a serious problem for the treatment of these infections given the limited choice of available antibiotics (linezolid, daptomycin, quinupristin/dalfopristin, and vancomycin) **(Georges *et al*., 2022)**; (ii) they have an impressive capacity to acquire new resistance genes due to the plasticity of their genome **(Aung *et al*., 2023)**.

Enterococcus spp., particulary *En. faecalis* and *En. faecium*, are the most opportunistic pathogens causing several infections, including medical device-associated infections, UTIs, wound infections, and BSIs **(Aung *et al*., 2023)**. *En. faecalis* is responsible for 80 to 90% of cases of Enterococci-associated NIs, followed by *En. faecium* (5 to 10% of infections) **(Farman *et al*., 2019; Boccella *et al*., 2021)**. **Putta *et al.* (2018)** revealed that *En. faecalis* was the most causative agent of CA-UTIs in ICU-patients. In another study, 13.11% of *En. faecium* were isolated from CA-UTIs **(Patel *et al*., 2021)**. Different rates were obtained in other studies: 7.1% **(Singh *et al*., 2018a)**, 91% **(Bahuleyan *et al*., 2019)**, 19% **(Gul *et al*., 2021)**, 22.9% **(Perdana *et al*., 2023)**, and 29% **(Nye *et al*., 2024)**. The presence of these pathogens in CA-BSIs was also reported **(Tomar *et al*., 2015; Awadh *et al*., 2021)**. *En. faecalis* was the 5th most frequently isolated bacterium from CA-UTIs and the 3rd from CLA-BSIs, unlike *En. faecium*, which was the 11th and 5th in CA-UTIs and CLA-BSIs, respectively **(Garcia-Solache *et al*., 2019)**.

However, in recent years the prevalence of infections due to *En. faecium* has increased, overtaking the prevalence of *En. faecalis*, and this is due to the emergence of antibiotic resistance, especially vancomycin-resistant *En. faecium* (VREfm), which is responsible for the most vancomycin-resistant *Enterococcus* (VRE) infections in the world **(Markwart *et al*., 2019;**

Ramos *et al*., 2020; Monteiro-Marques *et al*., 2023), thus leading the WHO to include VREfm in the list of high priority pathogens **(Monteiro-Marques *et al*., 2023)**. Of note, glycopeptides resistance in Enterococci is attributed to the acquisition of different clusters of genes (e.g. *van*A, *van*B, *van*D, *van*E, *van*G, and *van*L) which confer resistance **(Kresken *et al*., 2022; Smout *et al*., 2023)**.

In addition to antibiotic resistance, the ability of *Enterococcus* strains to form biofilms is one of the primary factors involved in their virulence and pathogenicity notably on medical devices **(Kim *et al*., 2020a; Khalil *et al*., 2022)**. Several investigations have been conducted on Enterococci virulence factors involved in biofilm formation. **Soares *et al*. (2014)** found that *En. faecalis* possessed *esp*, *gel*E, and *asa*1 genes. **Kafil and Mobarez (2015)** reported the presence of *esp*, *ebp*A, and *ebp*B genes found in high biofilm producers. Similar results were found in the study of **Khalil *et al.* (2022)**. It has been reported also that the biofilm-associated pilus (Ebp) is involved in biofilm formation on URC leading to CA-UTI **(Flores-Mireles *et al*., 2014; Kafil and Mobarez, 2015)**. Given that *En. faecalis* is the most identified species in biofilm-associated infections **(Shahriar *et al*., 2022; Woitschach *et al*., 2022)**, the virulence factors cited in **Table 2 (Appendix I)** only concern this bacterial species.

Chapter IV. Antimicrobial peptides: Promising agents to fight medical device-associated biofilm infections

1. Antimicrobial peptides and their characteristics

The lysozyme is the first human antimicrobial protein discovered from nasal mucus by Alexander Fleming in 1921 (Ferraboschi *et al*., 2021). This identification was quickly eclipsed with the discovery of penicillin in 1928 by the same researcher. Thus, in the 1940s, the therapeutic use of penicillin and other antibiotics marked the beginning of the "Golden Age of Antibiotics", leading to a loss of interest in the therapeutic characteristics of antimicrobial peptides. It was only in the 1960s that their interests were awakened due to the worldwide emergence of MDR- pathogens **(Di Somma *et al*., 2020a)**. Since that time, the number of peptides has surged significantly, hastening the development of AMP databases **(Zhang *et al*., 2023)**.

More than ten AMP databases have been established to classify AMPs so far, including APD3, DBAASPv3, CAMP3, dbAMP2, ANTI-MIC, YADAMP, LAMP2, DRAMP3.0, CyBase and PenBese. However, the first four are most well-known due to their large data resources and powerful function, thus attracting more users **(Gabere *et al*., 2017)**. Until 2023, a total of 3425 antimicrobial peptides are listed in the Antimicrobial Peptide Database (APD3) **(Zhang *et al*., 2023)**, of which 98% have been identified in nature. However, as of January 2024, the APD3 contains 3940 peptides, including 3146 natural AMPs (2463 from animals, 383 bacteriocins/peptide antibiotics from bacteria, 250 from plants, 29 from fungi, 8 from protists and 5 from archaea), 190 predicted and 314 synthetic AMPs. Among the natural AMPs with known antimicrobial activity, there are 144 human host defense peptides (https://aps.unmc.edu, consulted on 09/12/2024). Most of them are composed of positively charged and hydrophobic amino acids **(Mangmee *et al.*, 2021)**.

According to Databank antimicrobial peptides (dbAMP), AMPs could be divided into various categories such as antibacterial peptides (ABPs), antiviral peptides (AVPs), antifungal peptides (AFPs), anticancer peptides (ACPs) and antiparasitic peptides (APPs). In dbAMP, ABPs are the major AMPs with 35.6%, followed by AFPs (14.31%) **(Ramazi *et al.*, 2022)**.

Antimicrobial peptides (AMPs), called also host defense peptides, which are considered as a part of innate immune system of humans, animals, plants and microorganisms, have lately attracted the attention as promising alternative for infections treatment and as new immune-modulation therapies **(Mahlapuu *et al*., 2020; Ramazi *et al*., 2022; Duarte-Mata and Salinas-**

Carmona, 2023) and are less affected by antibiotic resistance mechanisms **(Akhash *et al*., 2024)**. AMPs exhibit broad spectrum activity against Gram- negative and Gram- positive bacteria as well as fungi **(Chung and Khanum, 2017)** and are more effective against Gram-negative bacteria unlike Gram- positive bacteria due to differences in cell wall composition **(Sahoo *et al*., 2021)**. In addition, the AMPs are also reported to have anti-biofilm function **(Zhang *et al*., 2023)**.

The natural AMPs have quite long sequences (12 to 80 amino acids), which makes their use as drugs difficult due to the high cost of producing proteins on an industrial scale **(Mikut *et al*., 2016; Cardoso *et al*., 2021)**. The natural AMPs are not stable with short half-life, toxic side effects and may have high hemolytic activity **(Lei *et al*., 2019)**. Moreover, many natural peptides showed rapid degradation by proteases, instability due to pH variations and decreased activity in the presence of biological fluids **(Vanzolini *et al*., 2022)**. Therefore, the research focuses on the development of synthetic peptides which represent promising new therapeutic agents which attract more and more attention because of their strong biomimetic mechanisms of action, their ease of synthesis and their low cost of production **(Mikut *et al*., 2016; Cardoso *et al*., 2021)**. In fact, synthetic peptides are an ideal alternative which have been developed from existing peptides and which have low toxicity for eukaryotic cells taking into account their amino acids composition, their charge, their hydrophobicity but also their antimicrobial characteristics **(Oliveira *et al*., 2019)**.

AMPs are able to target biological membranes (interaction between cationic peptides and anionic lipids such as LPS) and are active at concentrations of the order of micromolar. In addition, AMPs can also activate immune cells leading to microbial death and control inflammation **(Teixeira *et al*., 2020)**.

2. Structure of AMPs

AMPs are short peptides, composed of 15 to 50 amino acids, cationic with a net positive charge ranging from +2 to +9, due to the abundance of Arginine, Lysine, or Histidine residues which attracts them to the membranes of bacteria which are generally negatively charged via electrostatic forces. They are also composed of approximately 50% hydrophobic residues (e.g. Tryptophan, Phenylalanine, or Leucine) which allow them to fold into amphiphilic conformation upon interaction with bacterial membranes. This structure is important for their mechanism of action **(Pfalzgraff *et al*., 2018; Lazzaro *et al*., 2020; Clark *et al*., 2021)**. The net positive charge of the peptides facilitates their interactions with negatively charged bacterial membrane

phospholipids. Hydrophobicity also plays an important role in membrane permeabilization, gate formation and cell death **(Wang *et al*., 2019a)**. These peptides have N-terminus rich in basic to strong alkaline amino acids, and they are amidated at their C-terminus with neutral C-terminus hydrophobicity **(Lei *et al*., 2019)**. There are also anionic antimicrobial peptides. These peptides are rich in glutamic and aspartic acids, have a net charge at neutral pH of 1 to 7 and a length of 5 to 70 residues. Some of them require post-translational modifications in order to exhibit antimicrobial activity. The amphibian peptide Maximin-H5 and Dermcidin, a peptide derived from human sweat are representative examples of this group **(Cruz *et al*., 2014)**.

3. Classification of AMPs

3.1. Classification of AMPs based on sources

The antimicrobial peptides can be found in: mammals such as cattle, sheep and in particular in humans (called human host defense peptides "HDP"). HDPs can be found in different parts of the body (e.g. skin, eyes, ears, mouth, intestine and urethra) **(Huan *et al*., 2020)**. For example, LL-37 cathelicidin is usually detected in the skin of newborns, while human beta-defensin 2 (hBD-2) is often expressed in the elderly instead of the young **(Gschwandtner *et al*., 2014)**. Another example, lactoferricin B which is produced from lactoferrins, milk proteins of cattle **(Sibel Akalın, 2014)**; plants such as the defensin PaDef extracted from avocado, *Persea americana* **(Guzmán-Rodríguez *et al*., 2013)**, purothionins from wheat seeds **(Li *et al*., 2021a)**, hevein from rubber trees **(Loo *et al*., 2021)**; amphibian such as magainin and temporin derived from skin secretions of the species *Xenopus laevis* and *Rana temporaria* **(Wang *et al*., 2020)**; insect such as moricin and cecropin B isolated from the species *Bombyx mori* and *Hyalophora cecropia*, respectively **(Sahoo *et al*., 2021)**; snake such as the cathelicidin, CATHPb1, isolated from the Burmese python *Python bivittatus* **(Cai *et al*., 2018)**; and microorganisms such as nisin from *Lactococcus lactis*, subtilin from *Bacillus subtilis*, Gramicidin A from *Brevibacillus brevis* (formely named *Bacillus brevis*) and Plantaricin A from *Lactiplantibacillus plantarum* (formely named *Lactobacillus plantarum*) **(Cao *et al*., 2018)**.

3.2. Classification of AMPs based on their structures

Antimicrobial peptides are divided into four categories based on their structures: α-helical, β-sheet, extended, and loop peptides **(Figure 6)** (**Di Somma *et al*., 2020a**). α-helical peptides (e.g. LL-37, Magainin-2) are free of cysteine residues, linear in aqueous solution and adopt amphipathic helical structures when interacting with biological membranes. β-sheet peptides (e.g. ß-defensin-3, protegrin I) have more rigid structure due to the presence of two or

more disulfide bridges forming a stable structure in solution and do not need major structural changes upon interaction with the membranes. The extended peptides (e.g. Indolicidin) are linear and have no secondary structure, no cysteine and are rich in proline, arginine, glycine, and tryptophan **(Lewies *et al*., 2015; Di Somma *et al*., 2020a; Moretta *et al*., 2021)**. The last group is loop peptides (e.g. Thanatin) are characterized by their loop structure and stabilized by a single bond (disulfide, amide, isopeptide bonds) **(Powers and Hancock, 2003; Kawano *et al*., 2020)**.

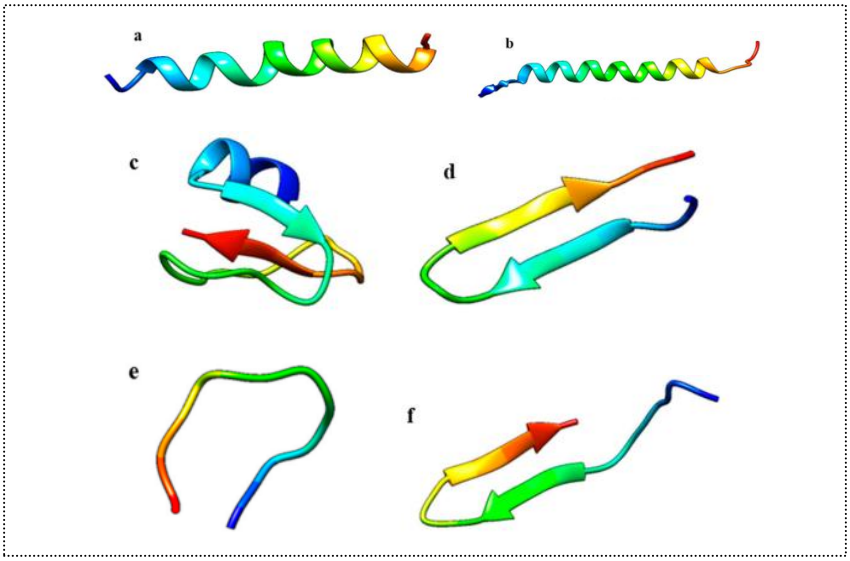


Figure 6. The structure of different classes of Antimicrobial peptides: α-helical, β-sheet, loop, and extended. (**a**) α-helical Magainin-2; (**b**) α-helical LL-37; (**c**) β-sheet Human β-defensin-3; (**d**) β-sheet Protegrin I; (**e**) extended Indolicidin; (**f**) loop Thanatin (**Di Somma *et al*., 2020a**).

4. Functions of AMPs

As mentioned above, many AMPs identified so far have a broad spectrum of biological activities, including antibacterial, anti-biofilm, anti-fungal, antiviral and anticancer activities **(Lei *et al*., 2019)**. In this synthesis, we will focus on the antibacterial, anti-biofilm and immunomodulatory activities.

4.1. Antibacterial activity

Antibacterial peptides have a broad inhibitory effect on several pathogenic bacteria, such as *A. baumannii*, MRSA and vancomycin resistant Enterococci which are responsible of NIs **(Huan *et al*., 2020)**. **Rivera-Sánchez *et al*. (2020)** recorded that the peptide cecropin D-like (ΔM2) had a strong effect on MDR strains (*K. pneumoniae* and *P. aeruginosa*) with a MIC and minimal bactericidal concentrations (MBC) between 4 and 16 µg/mL. **Zapotoczna *et al.* (2017)** demonstrated that the peptide D-Bac8c^{2,5Leu} alone or combined with antibiotics seem to be a

potential agent to treat *S. aureus* IVC-associated infections. Another Dutch study reported that SAAP-148 peptide seems to have an excellent antibacterial activity against MDR- ESKAPE pathogens **(de Breij *et al*., 2018)**. Other peptides including *polybia*-MPI **(Zhao *et al*., 2016)**, HYL-20k **(Nešuta *et al*., 2017)**, PS1-2 **(Park *et al*., 2019)**, Agelaia-MPI and Polybia-MPII **(das Naves *et al*., 2019)**, 3.1-PP4 **(Gomes *et al*., 2019)**, BP100-W **(Ajish *et al*., 2022)**, HG2 and HG4 (**Oyama *et al*., 2022)** and mKLK (derived from amidated sapecin B-derived peptide) **(Akhash *et al*., 2024)** showed a promising antibacterial activities towards Gram- negative and Gram-positive bacteria. The mechanism of the antibacterial activity of AMPs can be divided in two steps:

- **Crossing the outer membrane and cell wall of Gram-negative and Gram-positive bacteria**

First, cationic peptides interact with the negatively charged surfaces of Gram-negative (outer membrane) and Gram-positive (cell wall) bacteria. The outer membrane of Gram-negative bacteria includes LPS with negative charge. The initial association of peptides with the outer membrane occurs through electrostatic interactions between the peptide and LPS. These cationic peptides can also displace divalent cations such as Mg^{2+} and Ca^{2+} bound to LPS, causing the destabilization and disruption of this membrane **(Bechinger and Gorr, 2017; Lei *et al*., 2019)**. The anionicity of the surface of Gram-positive bacteria is due to the presence of peptidoglycan and teichoic acids. So the first contact of cationic peptides with these bacteria is by electrostatic interaction **(Omardien *et al*., 2016)**. In addition, the cell wall of these bacteria represents a porous mesh (40 to 80 nm thick) that many AMPs can easily pass through **(Malanovic and Lohner, 2016)**.

- **Membrane disruption**

Once the external barriers (outer membrane and cell wall) are crossed, cationic peptides (generally exhibit a balance between positively charged and hydrophobic amino acid residues which allows them to adopt an amphipathic conformation) bind to negatively charged phospholipids of the plasma membrane **(Malanovic and Lohner, 2016)** by electrostatic attraction, and the amphipathic peptide insert into the membrane interface. Then, many peptide molecules will insert into the membrane interface and aggregate as a micellar-like complex which extends over the membrane or else penetrate through the membrane under the influence of a gradient of transmembrane electrical potential **(Powers and Hancock, 2003)**. As a result, the

integrity of the bacterial cytoplasmic barrier is compromised of resulting in the leakage of cell contents and the cell death **(Da costa *et al*., 2015)**.

4.2. Anti-biofilm activity

In clinical settings, biofilms are considered to be a major cause of persistent NIs in hospitalized patients **(de la Fuente-Núñez *et al*., 2016; Roy *et al*., 2018)**. Although many studies on biofilms have been reported, there are still no antibiotics exclusively developed for this type of infections. In addition to being tolerant to antibiotics, biofilms are recalcitrant, which hinders their eradication and the effectiveness of conventional antibiotic therapy **(Silveira *et al*., 2021)**. Recent developments have provided evidence that the antimicrobial peptides constitute a promising approach to treat biofilm-associated infections **(Pletzer and Hancock, 2016)**. A number of peptides have demonstrated excellent anti-biofilm activity which can either inhibit or eradicate biofilms or both **(Andrea *et al*., 2018)**. Human cathelicidin LL-37 is considered one of the first AMPs with anti-biofilm activity against *P. aeruginosa* biofilm, showing an anti-biofilm activity at a concentration 16 times lower than its MIC through dysregulation of genes involved in the biofilm formation and/or mortality **(Overhage *et al*., 2008; Andrea *et al*., 2018)**. Other examples of anti-biofilm peptides are presented in **Table 2**. In a recent study, **Fei *et al.* (2023)** showed that the novel peptide called Brevinin-1E-OG9c-De-NH_2 exhibited a potent anti-biofilm activity against *S. aureus* strains. Another recent study demonstrated the potent inhibitory activity of mKLK against *S. aureus* biofilms in murine model of catheter-associated biofilm infection **(Akhash *et al*., 2024)**.

Regarding the AMPs mechanisms of action, several anti-biofilm peptides have been identified affecting multiple stages of the biofilm life cycle **(Hancock *et al*., 2021)**. For instance, the synthetic peptide 1037 suppressed swimming and swarming motility, which negatively affects the attachment of *P. aeruginosa* to surfaces. This peptide could also stimulate twitching motility, which can promote disassembly and the dispersion of the biofilm **(de la Fuente-Núñez *et al*., 2012)**. Another study reported that polymyxin B was able to inhibit the biofilm formation of *Vibrio cholerae* by disturbing the flagellar assembly **(Giacomucci *et al*., 2019)**. Other peptides can also interfere with the architecture of the biofilm by disrupting the extracellular matrix. For example, piscidin 3, a peptide identified in fish, could degrade eDNA, a component of the matrix (an amino-terminal motif in piscidin 3 binds to cationic copper, which leads to improved DNA binding and cleavage, thus enhancing anti-biofilm activity) **(Libardo *et al*., 2017)**. In addition, another anti-biofilm peptide HBD3 appears to be able to inhibit *Staphylococcus* biofilm formation by interfering with the expression of genes responsible for the

synthesis of the main extracellular polysaccharide PIA **(Zhu *et al*., 2013)**. **Pulido *et al*. (2016)** reported that the peptide RN3, a derivative of the cationic eosinophilic protein (ECP), was able to disrupt the membranes of *P. aeruginosa* cells within a biofilm.

As mentioned before, the stringent stress response, found in almost all bacteria, is triggered when bacterial cells are faced with stressors (limited nutrients, presence of reactive oxygen and/or nitrogen species, antibiotics) in their environment, which leads to the accumulation of a second messenger molecule called guanosine tetraphosphate (ppGpp), which promotes the formation of biofilm to cope with these conditions. Several anti-biofilm peptides such as IDR-1018, DJK-5 and DJK-6 have been reported to interfere with this response by binding to ppGpp preventing biofilm formation and maturation, as well as promoting biofilm dispersion **(de la Fuente-Núñez *et al*., 2014; de la Fuente-Núñez *et al*., 2015)**.

Table 2. Anti-biofilm peptides

Peptide	**Bacteria**	**Activity**	**Reference**
IDR-1018	*P. aeruginosa, E. coli* *A. baumannii, K. pneumoniae* MRSA	Inhibition/Eradication	**Mansour *et al*., 2015**
DJK-5 DJK-6	*P. aeruginosa, E. coli* *A. baumannii, K. pneumoniae*	Inhibition/Eradication	**de La Fuente-Núñez *et al*., 2015**
UP-5	MRSA	Inhibition	**Almaaytah *et al*., 2018**
SAAP-148	*A. baumannii, S. aureus*	Inhibition/Eradication	**de Breij *et al*., 2018**
3.1-PP4	*K. pneumoniae*	Inhibition	**Gomes *et al*., 2019**
Pep19-2.5, Pep19-4LF	*P. aeruginosa, S. aureus*	Inhibition	**Subh *et al*., 2020**
HG2, HG4	MRSA	Inhibition	**Oyama *et al*., 2022**
GHb3K, GHbK4R	*S. aureus*	Inhibition	**Jin *et al*., 2023**
NET1 NET3	*E. coli, S. aureus* MRSA, Vancomycin-resistant *Enterococcus*	Inhibition	**Polat *et al*., 2024**

4.3. Immunomodulatory activity

The peptides are an important part of the innate immune system acting as the first barrier against pathogens. In addition to their ability to kill microorganisms by disruption of membranes or inhibition of vital intracellular processes, the peptides are also able to interact with the host's

immune system to modulate its inflammatory responses **(Gan *et al.*, 2021)**. AMPs are able to recruit antigen presenting cells directly at the site of infection **(Browne *et al.*, 2020)**. They induce the differentiation of dendritic and T cells and activates them. They can suppress the expression of pro-inflammatory cytokines, induce the expression of anti-inflammatory cytokines, and thereby enhance phagocytosis **(Moravej *et al.*, 2018; Gan *et al.*, 2021)**. They can also promote wound healing by induction the migration of endothelial cells, their proliferation and increase angiogenesis **(Figure 4) (Pfalzgraff *et al.*, 2018)**.

In some cases, the body induces an unusually severe inflammatory response to infection, which can lead to sepsis which is a very serious problem and is often caused by LPS, also called endotoxin in Gram-negative bacteria and lipoproteins (LP) in Gram-positive bacteria **(Gan *et al.*, 2021)**. LPS is released during bacterial death **(Sun and Shang, 2015)**. The bacteria most frequently isolated from patients with sepsis include *S. aureus*, *Str. pneumoniae*, *E. coli*, and *P. aeruginosa* **(Nedeva *et al.*, 2019)**. Once LPS is released into the bloodstream, it is recognized by an LPS binding protein (LBP) which is responsible for identifying monocytes and LBP-LPS complexes will be formed. This protein transfers LPS to the CD14 cell surface receptor of monocytes or phagocytes, forming an LPS-CD14 complex to activate the cells. Then, the LPS-CD14 complex initiates intracellular signaling by interacting with a membrane protein Toll-like receptor 4 (TLR4) (TLR2 in Gram- positive bacteria). Once TLR4 is bound to LPS due to MD-2, it activates several different signaling molecules, such as nuclear factor κB (NF-κB), which in turn induce the secretion of pro-inflammatory cytokines (e.g. TNF- α, IL-1β, IL-6 and IL-8). Over-expression of these cytokines causes septic shock which rapidly leads to multiple organ damage and eventual death **(Gan *et al.*, 2021)**.

In the presence of peptides, they are able to bind to LPS and suppress the expression of TNF-α induced by LPS **(Sun and Shang, 2015)**. In fact, due to the negative charge of the polysaccharide core and the phosphate group of LPS, they can be strongly bound to cationic peptides. Therefore, the alkyl chains of LPS and the non-polar side chains of AMPs interact via hydrophobic interactions. Once bound to LPS, peptides can neutralize LPS and inhibit the release of inflammatory factors **(Luo and Song, 2021)**. Several peptides such as *Galleria mellonella* native peptide and their analogues (Gm1, ΔGm1) **(Correa *et al.*, 2014)**, D-RR4 **(Mohamed *et al.*, 2017)**, Pep19-2.5 **(Heinbockel *et al.*, 2018)**, Ps-K18 **(Jang *et al.*, 2019)**, BP100-W **(Ajish *et al.*, 2022)** and Brevinin-1E-OG9c-De-NH_2 **(Fei *et al.*, 2023)** have been shown to neutralize LPS and control inflammatory responses. These properties make the

antimicrobial peptides promising candidates for the treatment of endotoxin shock and sepsis caused by Gram-negative and Gram-positive bacterial infections **(Jang *et al*., 2019)**.

5. Combination therapy with antimicrobial peptides

Antibiotic resistance is a threat that poses a serious health hazard to people around the world with increasing cases of hospitalizations and deaths, making the development of new strategies very urgent **(Umerska *et al*., 2018; Browne *et al*., 2020)**. The use of AMPs in combination is considered as an effective strategy to overcome antibiotic resistance and toxicity **(Andersson *et al*., 2016; Sierra and Viñas, 2021)**. In fact, it has been reported that combined therapy with peptides could solve, at least the problem of antimicrobial resistance **(Marquette *et al*., 2018; Cheng *et al*., 2019)** because the simultaneous development of resistance to several antimicrobials is unlikely **(Pizzolato-Cezar *et al*., 2019)**. The advantage of treating infectious diseases caused by MDR bacteria and biofilm formation with combinations of antimicrobials is the fact that the mechanism of action of peptides by membrane permeabilization is completely different from conventional antibiotics **(Hollmann *et al*., 2018; Sen-Kilic *et al*., 2019)**. As we mentioned before, the peptides increase the fluidity of bacterial membranes, as well as their permeability, facilitating the absorption of other substances, including antibiotics, promoting a synergistic effect that can affect other targets inside the bacterial cell **(Hollmann *et al*., 2018; Armengol *et al*., 2019)**. The researchers have highlighted the synergistic effects of peptide and antibiotic combinations against multi-resistant bacteria **(Wu *et al*., 2021c)**. For instance, a recent publication highlighted the permeabilizing function of polymyxin B and its derivatives which damage the outer membrane of Gram-negative bacteria, making it more permeable to other antibiotics **(Vaara, 2019)**. It was also reported that the antibiotic-resistant bacteria exhibit increased collateral sensitivity to antimicrobial peptides and induction of cross-resistance is relatively rare **(Lázár *et al*., 2018)**. The same study showed also that co-administration of the antimicrobial peptide glycine-leucine-amide (PGLA) as an adjuvant decreased the level of antibiotic resistance by up to 30 times. All this evidence can be used for the development of combined therapies against multi-resistant pathogens and the decrease of the evolution of antibiotic resistance **(Lázár *et al*., 2018)**. However, the combined therapy may be accompanied with various disadvantages such as antagonism, super-infection, increased side effects and increased costs **(Hollmann *et al*., 2018)**. In addition, the design of new low or non-toxic antimicrobial peptides is a primary condition for therapeutic application. Although this cytotoxicity depends on their structure and their composition, it also strongly depends on their concentration **(Sierra and Viñas, 2021)**. Combined them with other peptides or with

conventional antibiotics allows the use of lower doses of each antimicrobial agent improving their effects, limiting harmful side effects *in vivo* and reducing host toxicity **(Greco *et al*., 2020; Duong *et al*., 2021)**.

Several studies reported the synergistic enhancement of peptides combined with other peptides **(Nishida *et al*., 2007; Zerweck *et al*., 2017 ; Bechinger *et al*., 2020; Xie *et al*., 2020)** and with conventional antibiotics **(Swedan *et al*., 2019; Wu *et al*., 2021a; Kalsy *et al*., 2020; Jahangiri *et al*., 2021; Sacco *et al*., 2022; Kim *et al*., 2023a; Prosswimmer *et al*., 2024)**. Drug combinations may result in synergy, additivity or antagonism; which means that the inhibitory effect of the two drugs combined is greater, equal or weaker than that of the drug alone, respectively **(Yu *et al*., 2016)**. Synergy is measured *in vitro* using the Fractional Inhibitory Concentration Index (FICI) using the checkerboard method to assess bacterial growth in the presence of different concentrations of two antibiotics. The FICI is defined as the sum of the MICs of each antibiotic combined divided by the MIC of the antibiotic alone **(Coates *et al*., 2020)**. In conclusion, the combination therapy constitutes a good way to improve the therapeutic action of AMPs.

Materials and Methods

1. Place of study

This study was carried out at the level of two laboratories: Applied Microbiology Laboratory (LMA), University A. Mira (Bejaia city, Algeria) and Laboratory of Department of Infectious Diseases, Leiden University Medical Center (LUMC) (Leiden, The Netherlands).

2. Origin of medical devices

Between February and April 2016, a total of 40 medical devices (28 intravenous catheters, 8 urinary catheters, and 4 intra-abdominal drains), taken from 39 patients who have stayed at least 48 h at the departments of Resuscitation, General Surgery, Internal Medicine and Infectious diseases of two hospitals and a private healthcare unit (Bejaia, Algeria), were analyzed.

3. Samples collection

3.1. Medical devices collection

The medical devices were collected under aseptic conditions. Pieces of 3 to 4 cm of each device were cut then placed individually in sterile boxes and transported in a cooler (4°C) immediately to the Applied Microbiology laboratory for analysis.

3.2. Urine collection

Seven urine samples were taken from catheterized patients. Urine collection was carried out according to **SPILF (2002)** under strict aseptic conditions. After disinfection of the tubing of the catheter, collection was done by clamping the excretion tube for 15 to 20 min **(Figure 7)**. Urine collects upstream and a puncture was performed. A volume of 5 to 10 mL of urine was collected in sterile boxes and was directly transported in a cooler (4°C) to the laboratory for analysis. Culture began within 2 h of collection. Each sample was accompanied by an information sheet including the sex, age, nature of the pathology that prompted the urinary catheterization, the type of catheter and the duration of catheterization **(SPILF, 2002)**.

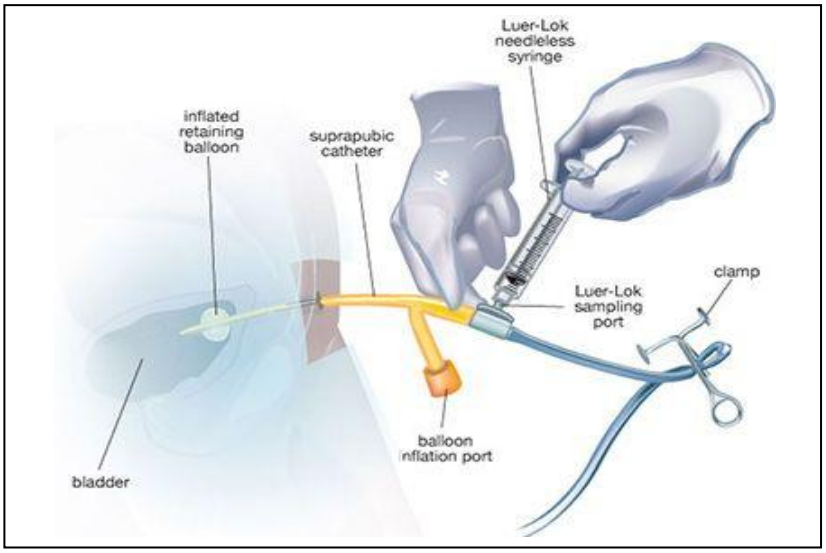


Figure 7. Picture indicating the site for collecting urine from a urinary catheter **(Kabir, 2022)**

4. Urine and medical devices analysis

4.1. Urine analysis

A cyto-bacteriological examination (CBE) was performed for each urine sample. This examination consisted on:

- **Macroscopic observation**: Visually, urine appearance (opacity, color) was investigated;

- **pH measurement**: 5 mL of urine were used for pH measurement (pH meter Hanna, Cluj Napoca, Romania);

- **Cytological examination**: A drop of urine was observed between slide and coverslip, under an optical microscope (magnifications 100x and 400x), and presence of epithelial cells and/or crystals was determined. An enumeration of red blood cells and leukocytes was also done using a Malassez slide;

-**Bacterial culture**: The bacterial enumeration was carried out by the poor plate method, on nutrient agar (NA, Conda, Madrid, Spain) and trypticase soy agar (TSA, Biokar Diagnostics, Allonne, France) from ten- fold urine dilutions made in salt water (0.9 % [v/v] NaCl) and following an incubation at 37°C for 24 h to 48 h.

4.2. Medical devices analysis

From the medical devices, 33/40 of them (insertion≤7 days) were subjected to bacteria enumeration and isolation. The seven remaining devices (insertion>7 days) were used only for biofilm diffusion assay.

4.2.1. Enumeration of the bacterial flora

The bacteria were harvested from the medical devices using the method of **Brun Buisson *et al*. (1987)** with minor modifications. For this purpose, the tip of the intravenous catheter, urinary catheter and a piece of the intra-abdominal drain were cut off, transferred to 10 mL of sterile tryptone salt (TS) solution (0.9% [w/v] NaCl + 0.1% [w/v] tryptone in water) and then sonicated for 5 min at 42 kHz (Ultrasonic Cleaner Bransonic, Saint-Louis, MIS, USA). The bacterial suspension was vortexed for 1 min. After that, a volume of 15 µL of each bacterial suspension was spread on nutrient agar (NA, Conda, Madrid, Spain) and trypticase soy agar (TSA, Biokar Diagnostics, Allonne, France) plates for enumeration of the total flora, on eosine methylene blue agar (EMB, Conda, Madrid, Spain) plates to assess *Enterobacteriaceae* counts and on mannitol salt agar (MSA, Liofilchem, Teramo, Italy) plates to assess *Staphylococcus* spp. counts. To verify the efficacy of the sonication, the devices were washed and resuspended in 10 mL of nutrient broth (NB, Conda, Madrid, Spain). After incubation at 37°C for 24-48 h, streak isolations were realized from each positive broth on the specific agar plates as mentioned above.

4.2.2. Bacterial isolation

The isolation of the bacterial strains was carried out by aspirating the sterile TS solution found in each box containing the medical device and then adding a volume of 10 mL of nutrient broth. After incubation at 37°C for 24-48 h, streaking isolations were carried out on specific agars from each positive broth as shown in **table 3** below.

Table 3. Incubation temperature of the agar plates depending on the target bacterium

Specific medium	Target bacteria
Trypticase Soy Agar (TSA)	Incubation at 37°C to isolate the total flora
Eosine Methylene Blue Agar (EMB)	Incubation at 37°C to isolate *Enterobacteriaceae* Incubation at 44°C to select *E. coli* and *Enterobacter* spp.
Mannitol Salt Agar (MSA)	Incubation at 37°C to isolate *Staphylococcus* spp.

4.2.3. Biofilm diffusion from medical devices

Biofilm diffusion from 7 medical devices was assessed by the roll-plate technique using the protocol of **Djeribi *et al*. (2012)**. Intravenous catheters, urinary catheters and intra-abdominal drains, taken from patients in whom they have been left *in situ* for more than 7 days, were aseptically and carefully cut into discs and small pieces. After that, 4 discs or pieces were placed

on the surface of Columbia (Liofilchem, Italy) and Trypticase Soy Agar (TSA, Biokar Diagnostics, Allonne, France) plates supplemented with 5 % (v/v) of blood then incubated at 37°C for 24 h, 48 h and 72 h.

5. Purification and preservation of isolates

After incubation, three to five characteristic colonies were taken from the plates on the basis of their macroscopic appearance (color, shape, diameter, opacity, etc.), then purified until very distinct and homogeneous pure colonies were obtained. The pure bacterial isolates were stored in nutrient broth supplemented with 20% glycerol at 4°C and -18°C.

6. Identification of bacterial strains

Bacteria were identified firstly by phenotypic tests (colony morphology, Gram staining, respiratory mode, catalase and coagulase tests, mini-biochemical galleries) at the Applied Microbiology Laboratory, then genotypically (16S rDNA sequencing) at the Laboratory of Department of Infectious Diseases, Leiden University Medical Center (LUMC).

6.1. Phenotypic identification

6.1.1. Morphological study

The identification of the strains was carried out by referring to macroscopic examination (appearance of colonies on specific medium, growth temperature) and microscopic examination (Gram staining, shape and cellular organization).

6.1.2. Biochemical study

Each isolate was cultured in nutrient broth and then 24-h cultures were used for the different identification tests (classical tests, minimal biochemical gallery: API 20E and APIStaph **(Appendix II)**.

6.1.3. Identification of *Enterobacteriaceae* and *Acinetobacter* spp.

Preliminary identification of *Enterobacteriaceae* and *Acinetobacter* spp. isolates was performed by using a minimal biochemical gallery including the following media: TSI (Conda, Madrid, Spain), Urea–Indole (Prepared at the laboratory), MR-VP broth (Himedia, Mumbai, India), Simmons citrate (Himedia, Mumbai, India), Nitrate broth (Himedia, Mumbai, India) and mannitol motility (Himedia, Mumbai, India). Catalase production and respiratory mode were also tested. The isolates were further characterized by the sugar fermentation pattern, which was determined using the API 20E gallery, as specified by the manufacturer (BioMerieux, Marcy-l'Etoile, France) **(Appendix II)**. All of the key biochemical tests, performed for the

identification of Gram-negative bacteria (*Enterobacteriaceae* and *Acinetobacter* spp.) strains, are described below.

- **Catalase production**

The test was performed according to **Khatoon *et al*. (2022)**. For this, bacteria were mixed with H_2O_2. If bubbles appear (due to the production of oxygen gas) the bacteria are catalase positive; whereas if no bubbles appear, the bacteria are catalase negative.

- **Respiratory mode type**

The test was performed according to **Guetarni *et al*. (2024)**. Meat-liver semi solid agar (Biokar Diagnostic, Allone, France) was used to identify the respiratory type of bacteria; the tubes were inoculated by central stabbing and incubated at 37°C for 24-48 h. Four main types of respiratory mode can be recognized: strict aerobes, which develop only in the superficial zone; strict anaerobes, which develop only at depth; facultative aero-anaerobes, which develop along the top of the medium; micro-aerobes, which form a ring in the intermediate aerobiosis-anaerobiosis zone.

- **Motility and Mannitol fermentation**

The test was performed according to **Shields and Cathcart (2011)**. For this, the semi solid medium Mannitol-Motility Agar (Himedia, Mumbai, India) was used; the tubes were inoculated with a pure culture by central stabbing and incubated at 37°C for 24 h. Mannitol positive culture means the Mannitol is fermented and the medium will turn to yellow; a positive motility test is indicated by a diffuse cloud of growth away from the line of inoculation.

- **Glucose, Lactose and/or Sucrose fermentation, gas and hydrogen sulfide production**

The test was performed according to **Lehman (2005)**. For this, Triple Sugar Iron (TSI, Conda, Madrid, Spain) agar was used and divided into tubes in a slanted position; this test differentiates bacteria based on their fermentation of lactose, glucose and sucrose and on the production of hydrogen sulfide. TSI slant was inoculated by first stabbing the butt down to the bottom, the needle was withdrawn, and then the surface of the slant was streaked and then incubated at 37°C for 24 h. Yellow butt (acidification of the medium) means that glucose has been fermented; yellow slant (acidification of the medium) means that lactose and/or sucrose has been fermented; bubbles in the butt means gas production and blackening of the butt means the hydrogen sulfide production from sodium thiosulfate.

- **Urease activity and Indole production**

The test was performed according to **Rosa Fraile *et al*. (1980)**. For this, Urea- Indole medium (prepared at the laboratory) was used. The medium was inoculated by the target bacteria then incubated at 37°C for 24 h. Urease activity is observed by a change of color to red and Indole production is observed by the formation of a pink to red ring after the addition of 3 to 4 drops of Kovacs' reagent.

- **Citrate utilization as carbon and energy source**

The test was performed according to **Mac Williams (2009)**. For this, Simmons Citrate Agar (Himedia, Mumbai, India) was used and divided into tubes in a slanted position. This medium was inoculated by streaks on the surface of the slant and incubated at 37°C for 24 h; screw caps were placed loosely on the tube. If Citrate is used, growth will be visible on the slant surface and the medium will be an intense Prussian blue, whereas, if the Citrate is not used, trace or no growth will be visible, no color change will occur and the medium will remain the deep forest green color of the uninoculated agar.

- **Glucose fermentation pathways (Methyl Red and Voges-Proskauer tests)**

The test was performed according to **Mc Devitt (2009)**. For this, a tube of Clark Lubs broth (Himedia, Mumbai, India) was inoculated from a fresh pure culture of the test culture and incubated 37°C for 24-48 h. After incubation, for Methyl Red (MR) test, 2 mL of culture were transferred into a new sterile culture tube then 5 drops of the methyl red reagent were added. For Voges-Proskauer (VP) test, 0.6 mL of Barritt's reagent A (VP1 reagent, α-naphtol solution) and 0.2 mL of Barritt's reagent B (VP2 reagent, Potassium hydroxyde solution) were added into the remaining 2.5 mL of culture grown in Clark Lubs broth. MR-positive culture shows a red coloration as a result of high acid production and a decrease in the pH of the culture medium. MR-negative culture has a yellow color indicating a less acidic medium. VP-positive culture shows red coloration on top of the culture which means the bacteria fermented glucose via the butanediol pathway and produced acetoin. VP-negative culture has a yellowish color.

- **Nitrate reduction**

The test was performed according to **Buxton (2011)**. For this, Nitrate reduction test is carried out on the Nitrate Broth (Himedia, Mumbai, India). The tubes were heavily inoculated with a fresh culture of the strain and incubated at 37°C for 24 h. After incubation, 2 drops of NIT1 (α-naphthylamine) and 2 drops of NIT2 (sulfanilic acid) were added into the tube containing culture to be tested, mixed and left for few minutes. Nitrate reductase positive culture shows a change in the medium color to red after adding the reagents A and B. Nitrate reductase

negative culture don't show any color change after adding the reagents A and B followed by a red color after adding a small amount of zinc.

6.1.4. Identification of *Staphylococcus* spp.

Each isolate was cultured in nutrient broth and then 24-h cultures were used for the different identification tests. The isolates were further characterized by the sugar fermentation pattern, which was determined using the API Staph gallery, as specified by the manufacturer (BioMerieux, Marcy-l'Etoile, France) **(Appendix II)**. All of the key biochemical tests performed for the identification of *Staplycococcus* spp. strains are summarized in the **table 4**.

Table 4. Main biochemical tests performed for the identification of *Staphylococcus* spp.

Test	Description	Result
Catalase production **(Khatoon *et al*., 2022)**	As described for Gram-negative bacteria	As described for Gram-negative bacteria
Respiratory mode type **(Guetarni *et al*., 2024)**	As described for Gram-negative bacteria	As described for Gram-negative bacteria
Coagulase test **(Kateete *et al*., 2010)**	Colonies of test isolates were inoculated in 5 mL of brain heart infusion (BHI) broth, Pasteur Institute, Algeria), incubated at 37°C for 24 h, and then 0.5 mL of the culture were mixed with 0.5 mL of human plasma in sterile glass test-tubes then incubated at 37°C for 18 h. Observation was carried out after 1 h, 2 h, and 4 h then after 18 h of incubation.	- Plasma clotting indicates a positive reaction: Coagulase positive strain

6.1.5. Identification of *Enterococcus* spp.

Each isolate was cultured in nutrient broth and then 24 -h cultures were used for the different identification tests. All of the key biochemical tests performed for the identification of *Enterococcus* spp. strains are summarized in the **table 5**.

Table 5. Main biochemical tests performed for the identification of *Enterococcus* spp.

Test	Description	Result
Catalase **(Khatoon *et al*., 2022)**	As described for Gram-negative bacteria	As described for Gram-negative bacteria
Respiratory mode type **(Guetarni *et al*., 2024)**	As described for Gram-negative bacteria	As described for Gram-negative bacteria
Salt tolerance **(Harwood *et al*., 2004)**	Pure strains were inoculated in 5 mL of de Man, Rogosa and Sharpe (MRS) broth (Conda, Spain) containing 6.5% (w/v) sodium chloride (NaCl) then incubated at 37°C for 24 h.	
Growth at pH 9.6 **(Harwood *et al*., 2004)**	Pure strains were inoculated in 5 mL of MRS broth (Conda, Spain) adjusted to pH 9.6 then incubated at 37°C for 24 h.	Cloudy medium indicates bacterial growth
Resistance to 63°C/30 min **(Vu *et al*., 1999)**	A tube containing 5 mL of nutrient broth inoculated with a pure strain was placed in a water bath at 63°C for 30 min then cooled in ice water and finally incubated at 37°C for 24 h.	
Resistance to bile and Esculin hydrolyse **(El-Gendy *et al*., 2021)**	The isolates were cultured on Bile Esculin Agar (BEA, Himedia, Mumbai, India) and incubated at 37°C for 24 h.	-Dark brown or black colonies on the agar plate are presumed as Enterococci isolates. Enterococci are able to grow in the presence of bile and hydrolyze Esculin to Esculetin that reacts with ferric citrate.

6.2. Genotypic identification (16S rDNA sequencing)

6.2.1. DNA extraction

The bacterial isolates were genotypically identified by 16S rDNA sequencing. Briefly, single colonies were obtained from the NA plate using a sterile plastic disposable loop and

bacterial DNA was extracted using 20 µL of lysis buffer [0.25 % (w/v) SDS, 0.05 N NaOH] and heating for 15 min at 95°C. The DNA samples were rapidly cooled down and centrifuged at 13,000 rpm for few seconds (Beun De Ronde, Abcoude, the Netherlands). Next, 180 µL of milliQ water were added and the DNA solution was mixed thoroughly, centrifuged again for 5 min at 13,000 rpm, and stored at -20°C until use.

6.2.2. Electrophoresis on agarose gel and sequencing

A PCR was used to amplify approximately 1,500 bp of a consensus 16S rDNA gene: forward primer αβ NOT (5'-AGT TTG ATG CTG GCT CAG-3') and reverse primer ω MB (5'-TAC CTT GTT ACG ACT TCG TCC CA -3'). Two µL of DNA sample, 2 µL of 10 µM forward and reverse primers, 25 µL of Master mix (Promega, Madison, WI, USA) **(Table 6)** and 21 µL of milliQ water were mixed and subjected to the following program: 1x5 min at 95°C, 3x1 min at 95°C, 2 min at 50°C, 1 min at 72°C, 35x (45 sec at 95°C, 45sec at 50°C, 1 min at 72°C) and 1x5 min at 72°C. Amplified products were analyzed by electrophoresis (Bio-Rad, Hercules, CA, USA) on 1% (w/v) agarose (Roche Diagnostics, Mannheim, Germany) gel at 120 V for 2 h. The PCR product was cleaned as follows: 0.5 µL of *ExoI* (20 units/µL; Thermo Scientific, Vilnius, Luthuania) and 1 µL of *Fast AP* (1 unit/µL; Thermo Scientific) were added to 5 µL of PCR product and incubated at 37°C for 15 min. The enzymes were then inactivated by heating at 85°C for 15 min and the mixture was finally diluted 10x with MilliQ water. For sequencing, 5 µL of PCR product, 1 µL of forward and reverse primers (1 pmol/µL) and 4 µL of MilliQwater were mixed. The sequencing was performed on Applied Biosystems 96-capillary system, ABI3730xl with a pop7 matrix (Macrogen, Amsterdam, the Netherlands).

Table 6. Master mix for PCR amplification

Composition	Concentration
Taq DNA polymerase	50 units/mL
dATP	400 Mm
dCTP	400 µM
dGTP	400 µM
dTTP	400 µM
$MgCl_2$	3 mM

Taq DNA polymerase was supplied in a proprietary reaction buffer (pH 8.5), dATP: deoxyadenosine triphosphate, dCTP: deoxycytidine triphosphate, dGTP: deoxyguanidine triphosphate, dTTP: deoxythymidine triphosphate.

7. Biofilm formation on polystyrene plates

Biofilm formation on sterile flat-bottom 96-wells polystyrene microplates (Greinerbio-one, Frickenhausen, Germany) was assessed using the TCP (Tissue Culture Plate) method as described by **O'Toole and Kolter (1998)**. The wells of the microplate, previously filled with 100 µL of trypticase soy broth (TSB, Biokar Diagnostics, Allonne, France), were seeded in triplicate with 100 µL of an 18 -h bacterial culture ($1x10^6$ CFU/mL) and incubated at 37°C for 24 h. Wells containing 200 µL of sterile TSB broth were included as negative controls. After incubation, the cultures were removed, the wells washed carefully with 200 µL of sterile TS solution and the biofilms fixed with 200 µL absolute ethanol (Biochem-Cheopharma, Montreal, Quebec, Canada). Subsequently, the fixed biomass was washed, then stained with 0.1% (w/v) crystal violet solution (Biochem-Chemopharma, Montreal, Quebec, Canada) for 30 min and finally washed with 200 µL of TS solution. The biomass was finally quantified by measurement of the absorbance of crystal violet extracted with 96% (v/v) ethanol at 630 nm using a microplate reader (Chromate Awareness Technology, Palm City, FL, USA). The classification of the strains on their adhesion capacity is presented in **table 7**.

Table 7. Interpretation of absorbencies in the TCP method **(Stepanovic *et al*., 2000)**.

Non adherent strain	Weakly adherent strain	Moderately adherent strain	Strongly adherent strain
OD≤ODc (control)	ODc<OD≤2xODc	2xODc<OD ≤ 4xODc	4xODc<OD

OD: Optical density, ODc: Optical density of the control

8. Slime production on Congo Red Agar (CRA)

The phenotypic characterization of the biofilm formation ability by slime production was carried out by culturing the isolated strains on congo red agar (CRA) as described by **Freeman *et al.* (1989)**. The solid medium consisted of a brain heart infusion broth (BHIB, Pasteur Institute, Algeria) supplemented with 5 % (w/v) sucrose (Biochem, Quebec, Canada), 0.08 % (w/v) congo red (Biochem, Quebec, Canada) and 1 % (w/v) of agar (Titan Biotech LTD, Rajasthan, India). The strains were inoculated on the CRA plates and incubated at 37°C/24 h. Strong slime producers were indicated by black colonies with a dry crystalline consistency. Weak slime producers usually remained pink, though occasional darkening at the centers of colonies was observed. Non slime producers were indicated by red colonies with a smooth surface **(Mathur *et al*., 2006)**.

9. Detection of bacterial virulence and adhesion genes involved in biofilm formation

The detection of virulence and adhesion genes by the clinical isolates was assessed by PCR. Briefly, 50 µL of buffer containing bacterial DNA, 25 µL of Master Mix [Taq polymerase, polymerase buffer (pH=8.5) and 400 µM dNTPs2x (Promega)], 10 µM forward and reverse primers **(specific primers in tables 8A-B-C)** were mixed; the PCR programs are indicated in **table 9A-B-C.**

Table 8A. Virulence and adhesion genes targeted and primers used for Gram- negative strains.

Bacterium	Gene	Gene function	Forward primer	Reverse primer	Product (bp)	Reference
E. coli	*Fim*H	Adhesins	TGCAGAACGGATAAGCCGTG	GCAGTCACCTGCCCTCCGGTA	508	**Yun *et al.*, 2014**
	*Csg*A	Curli adhesin	ACTCTGACTTGACTATTACC	AGATGCAGTCTGGTCAAC	200	**Pal and Singh, 2007**
	*Hly*F	Putative hemolysin	TCGTTTAGGGTGCTTACCTTCAAC	TTTGGCGGTTTAGGCATTCC	444	**Moulin-Schouleur *et al.*, 2007**
Klebsiella pneumoniae	*mrk*D	Adhesin type 3 *fimbriae*	AAGCTATCGCTGTACTTCCGGCA	GGCGTTGGCGCTCAGATAGG	340	**Compain *et al.*, 2014**
	*fim*H-1	Adhesin type1	ATGAACGCCTGGTCCTTTGC	GCTGAACGCCTATCCCCTGC	688	**Shah *et al.*, 2017**
	*ycf*M	Outer membrane lipoprotein	ATCAGCAGTCGGGTCAGC	CTTCTCCAGCATTCAGCG	160	**El fertasAissani *et al.*, 2013**
	*ecp*A	*E. coli* common pilus	AATGGTTCACCGGGACATCATGTC	AAGGATGAAATATCGCCGAC ATCC	759	**Cruz-Cordova et *al.*, 2014**
***Acinetobacter* spp.**	*csu*E	Adhesins	ATGCATGTTCTCTGGACTGATGTT GAC	CGACTTGTACCGTGACCGTAT CTTGATAAG	976	**Liu *et al.*, 2016**
	*omp*A	efflux system	CAATTGTTATCTCTGGAG	ACCTTGAGTAGACAAACGA	966	
	bap	biofilm-associated protein	TAG GGA GGG TAC CAA TGC AG	TCA TGA TTT GAT GCT GCA GCG ATA A	400	**Fallah *et al.*, 2017**

Table 8B. Virulence and adhesion genes targeted and primers used for *Staphylococcus* spp. strains.

Bacterium	Gene	Gene function	Forward primer	Reverse primer	Product (bp)	Reference
S. aureus	*ica*A	Intercellular adhesion protein A	CTTGCTGGCGCAGTCAATAC	CCAACATCCAACACATGGCA	178	**Zhang *et al*., 2018**
	*ica*D	Intercellular adhesion protein D	CGCTATATCGTGTGTCTTTTGGA	TCGCGAAAATGCCCATAGTT	164	
	*fnbp*A	Fibronectin protein A	ACCGTCAAACGCAACACAAG	TTCTGATGCCGTTCTTGGCT	259	**Chen *et al* 2020**
	*clf*A	Clumping factor A	ATTGGCGTGGCTTCAGTGCT	CGTTTCTTCCGTAGTTGCATTTG	288	
	*sas*G	Accumulation-associated protein	ACCACAGGGTGTAGAAGCTAAATC	CGAGCTTTTCTAACCTTAGGTGTC	189	**Reiter *et al*., 2012**
	Hlb	Hemolysin	GCCAAAGCCGAATCTAAG	CGCATATACATCCCATGGC	834	**Wang *et al*., 2016**
Coagulase negative *Staphylococcus*	*ica*A	Intercellular adhesion protein A	ACAGTCGCTACGAAAAGAAA	GGAAATGCCATAATGACAAC	103	**Srednik *et al*., 2017**
	*ica*D	Intercellular adhesion protein D	CGCTATATCGTGTGTCTTTTGGA	TCGCGAAAATGCCCATAGTT	164	**Zhang *et al*., 2018**
	*fnbp*A	Fibronectin protein A	CCCTCTTCGTTATTCAGCC	CAGGAGGCAAGTCACCTTG	422	**Kitti *et al*., 2019**
	embp	Fibronectin adhesion	AGCGGTACAAATGTCAAT	AGAAGTGCTCTAGCATCATCC	455	**Srednik *et al*., 2017**
	aap	Accumulation-associated protein	GAAGCACCGAATGTTCCAACTATC	AGTTGGCGGTATATCTATTGTA	289	**Tremblay *et al*., 2013**
	hlb	Hemolysin	TGGTGGCGTTGGTATTGTGA	ACCCCAAGATTTCACGGACC	541	**Nasaj *et al*., 2020**

Table 8C. Virulence and adhesion genes targeted and primers used for *Enterococcus* spp. strains.

Bacterium	Gene	Gene function	Forward primer	Reverse primer	Product (bp)	Reference
***Enterococcus* spp.**	*asa1*	Aggregation substance	GCACGCTATTACGAACTATATGA	TAAGAAAGAACATCACCACGA	375	**Vankerckhoven *et al*., 2004**
	esp	Extracellular Protein	GGAACGCCTTGGTATGCTAAC	GCCACTTTATCAGCCTGAACC	95	
	*gel*E	Gelatinase	TATGACAATGCTTTTTGGGAT	AGATGCACCCGAAATAATATA	213	

Table 9A. PCR programs used for the detection of virulence genes of Gram- negative strains

Target strain	Gene	Initial denaturation temperature (time)	Denaturation temperature (time)	Annealing temperature (time)	Extension temperature (time)	Number of cycle	Final extension temperature (time)	Final concentration (µM)
E. coli	*fim*H	94°C (5 min)	94°C (60s)	55°C (60s)	72°C (2min)	35	72°C (10min)	0.4
	*csg*A	95°C (3 min)	94°C (1min)	48°C (1min)	72°C (1min)	30	72°C (5min)	
	**hly*F	94°C (3 min)	94°C (1 min)	51°C (60s)	72°C (1 min)	30	72°C (10 min)	
K. pneumoniae	**mrk*D	95°C (5min)	94°C (30s)	60°C (90s)	72°C (1min)	30	72°C (10min)	0.1
	**fim*H-1							
	*ycf*M	95°C (4min)	95°C (45s)	55°C (1min)	72°C (1min)	30	72°C (5min)	
	**ecp*A							
***Acinetobacter* spp.**	*csu*E	95°C (10min)	95°C (30s)	63°C (30s)	72°C (1min)	35	72°C (10min)	0.4
	*omp*A	95°C (10min)	95°C (30s)	52°C (30s)	72°C (1min)	35	72°C (10min)	
	bap	95°C (2min)	95°C (30s)	61°C (30s)	68°C (1min)	30	68°C (2min)	

*PCR program was modified.

Table 9B. PCR programs used for the detection of virulence genes of *Staphylococcus* spp. strains

Target strain	Gene	Initial denaturation temperature (time)	Denaturation temperature (time)	Annealing temperature (time)	Extension temperature (time)	Number of cycle	Final extension temperature (time)	Final concentration (µM)
S. aureus	*ica*A	94°C (5 min)	94°C (30s)	55°C (30s)	72°C (1 min)	26	72°C (10min)	0.4
	*ica*D	94°C (5 min)	94°C (30s)	55°C (30s)	72°C (1 min)	26	72°C (10min)	
	*fnbp*A	94°C (5 min)	94°C (1min)	55°C (1min)	72°C (1 min)	25	72°C (5min)	
	*clf*A	94°C (5 min)	94°C (1min)	55°C (1min)	72°C (1 min)	25	72°C (5min)	
	*sas*G	94°C (5 min)	94°C (1min)	54°C (1min)	72°C (1min)	35	72°C (5min)	
	hlb	94°C (3 min)	94°C (30s)	55°C (30s)	72°C (1min)	30	72°C (5min)	
Coagulase negative *Staphylococcus*	**ica*A	94°C (5 min)	94°C (30s)	55°C (30s)	72°C (30s)	35	72°C (1 min)	0.4
	*ica*D	94°C (5 min)	94°C (30s)	55°C (30s)	72°C (1 min)	26	72°C (10min)	
	*fnbp*A	94°C (4 min)	94°C (1min)	58°C (1min)	72°C (1 min)	30	72°C (5min)	
	embp	94°C (5 min)	94°C (30s)	54°C (30s)	72°C (30s)	30	72°C (1 min)	
	aap	94°C (5 min)	94°C (30s)	54°C (30s)	72°C (30s)	30	72°C (1 min)	
	hlb	94°C (4 min)	94°C (1min)	58°C (1min)	72°C (1 min)	30	72°C (5min)	

*PCR program was modified.

Table 9C. PCR programs used for the detection of virulence genes of *Enterococcus* spp. strains

Target strain	Gene	Initial denaturation temperature (time)	Denaturation temperature (time)	Annealing temperature (time)	Extension temperature (time)	Number of cycle	Final extension temperature (time)	Final concentration (µM)
***Enterococcus* spp.**	*esp*	94°C (10 min)	94°C (1 min)	58°C (1min)	72°C (1 min)	35	72°C (10min	0.4
	*gel*E	94°C (10 min)	94°C (1 min)	56°C (1min)	72°C (1 min)	35	72°C (10min	
	*asa*1	94°C (10 min)	94°C (1 min)	52°C (1min)	72°C (1 min)	35	72°C (10min	

10. Antibiotic susceptibility testing

Antibiotic susceptibility testing of the strains was performed using the disc diffusion method. Briefly, bacterial suspensions of 0.5 McFarland which corresponds to an *inoculum* of approximately 10^8 CFU/mL were seeded on Mueller Hinton agar (Becton Dickinson, Mississagua, Ontario, Canada) plates, as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The plates were air-dried for 15 min and then discs impregnated with antibiotics (Becton Dickinson, Mississagua, Ontario, Canada) were deposited on the plates. The diameter of the inhibition zone around each disc was measured after 24 h at 37°C and the strains were graded as sensitive (S), intermediate (I) or resistant (R) following the **EUCAST (2017)** and the Clinical and Laboratory Standards Institute **(CLSI, 2017)**. *Escherichia coli* ATCC25922 was included as reference strain for quality control. Antibiotics tested are indicated in **table 10**.

Table 10. Antibiotics tested in this study

Bacterial species	Antibiotic	Concentration (µg)
E. coli ***K. pneumoniae***	Amoxicillin+clavulanic acid	20+10
	Cefotaxime, Ceftazidime, Cefoxitin, Cefepim, Tetracycline, Nalidixic acid	30
	Imipinem, Gentamicin	10
	Ciprofloxacin	5
	Co-trimoxazole	25
***Acinetobacter* spp.**	Cefotaxime, Ceftazidime, Cefepim, Tetracycline	30
	Imipinem, Gentamycin	10
	Ciprofloxacin	5
	Co-trimoxazole	25
***Staphylococcus* spp.**	Cefoxitin, Erythromycin, Tetracycline, Ceftarolin	30
	Gentamycin, Tobramycin	10
	Ciprofloxacin, Rifampicin	5
	Co-trimoxazole	25
En. faecalis	Tetracycline, Imipenem	30
	Ciprofloxacin, Rifampicine	5
	Erythromycin	15
	Co-trimoxazole	25

11. Extended Spectrum β-Lactamases (ESBL) production

The *Enterobacteriaceae* strains that showed resistance to at least one of the third cephalosporins were selected for checking the ESBL production **(Harwalkar *et al*., 2013)**. The ESBL production was tested by the Double Disc Synergy Test (DDST) by using a disc of amoxicillin-clavulanate (20/10 µg) along with ceftazidime (30 µg), cefotaxime (30 µg), cefepime (30 µg) and cefoxitin (30 µg) discs. The discs of ceftazidime, cefotaxime and cefepime were deposited at a distance of 20 mm (centre to centre) from the amoxicillin-

clavulanate disc on a Mueller-Hinton agar plate cultured by the target bacterium, as recommended by the EUCAST. The plates were incubated at 37 °C overnight. The presence of an ESBL results in the appearance of an image of synergy between the oxymino-β-lactam discs and the amoxicillin-clavulanate disc **(Jarlier *et al*., 1988)**.

12. Epsilometer test (E- test) vancomycin

Epsilometer test is an exponential gradient diffusion method of determination of antimicrobial susceptibility of microorganisms using E-test strips of desired antibiotic. This test was assessed to know the susceptibility of *En. faecalis* towards vancomycin. Briefly, bacterial suspensions of 0.5 McFarland were seeded on Mueller Hinton agar (Oxoid, Basingstoke, UK). The plates were air-dried for 5 to 15 min and then the strips (BioMérieux, Marcy l'Étoile, France) were placed on the plates and incubated for 24 h at 37°C. The MIC value is read at the point where the ellipse intersects the scale. The strains were graded as sensitive (S), intermediate (I) or resistant (R) following the **CLSI (2017**). *Escherichia coli* ATCC25922 was included as reference strain for quality control.

13. Hemolytic activity

Study of the pathogenicity of the strains was investigated by the hemolytic activity test on a Columbia agar (Liofilchem, Italy) supplemented with 5 % (v/v) of human blood as indicated by **Maragkoudakis *et al*. (2009)**. After incubation for 24-48 h at 37°C, the plates were examined. Three patterns of hemolysis can be observed on a blood agar plate. Alpha (α)-hemolysis (areas with greenish reflections around the colony) means incomplete lysis of red blood cells (RBC), beta (β)-hemolysis (clear areas around the colony) means complete lysis of RBC and gamma (γ)-hemolysis (No change in the blood agar around the colony) means absence of hemolytic activity.

14. Surface hydrophobicity: Bacterial adhesion to hydrocarbons

Surface hydrophobicity assay was performed as described by **Rosenberg *et al.* (1980).** The isolated strains were grown at 37°C for 18 h in NB. After centrifugation (8000 x *g*, 15 min, 4°C), the cells were washed twice with Phosphate Buffered Saline (PBS, 20 mM, pH= 7) and resuspended in the same buffer. The absorbance ($\mathbf{A_0}$) was measured at 600 nm in order to standardize the number of bacteria (~10^8 CFU/ mL), as measured by a spectrophotometer (UV-mini 1240 Shimadzu, Kyoto, Japan). A volume of 3 mL of the bacterial suspensions was

placed in tubes, and 1 mL of xylene (Sigma-Aldrich, Saint Louis, USA) was added. The tubes were then mixed with a vortex for 90 sec and allowed to stand (15 min) until the phases separated. After that, absorbance of the aqueous phase (1 mL) (**A**) was recorded. The percentage of hydrophobicity was calculated according to the formula:

$$\textit{Hydrophobicity (H) \%} = (1 - A/A_o) \times 100$$

Where A_0 represented the absorbance of the suspension before mixing with the solvent; A the absorbance of the aqueous phase after formation of the two-phases system.

The bacterial strains were classified as: non-hydrophobic strains (*H %* <20 %), moderately hydrophobic strains (20% < *H%* <50 %), and strongly hydrophobic strains (*H%* >50 %) **(Bellon-Fantaine *et al*., 1996)**.

15. Aggregation assay

Auto-aggregation and co-aggregation of the Gram-negative bacteria with the Gram-positive bacteria were assessed.

Aggregation assay was performed as described by **Kos *et al.* (2003)**. Bacterial suspensions were made as described above. Bacteria grown in NB were harvested by centrifugation (8000 x *g*, 15 min, 4°C), then washed with PBS (20 mM, pH= 7) and resuspended in the same buffer. For auto-aggregation assay, the cells suspensions (10 mL) were vortexed for 10 sec and then the absorbance (UV-mini 1240 Shimadzu, Kyoto, Japan) was measured at 600 nm ($\mathbf{A_0}$). The determination of self-aggregation was measured after 2 h of incubation at 37°C by taking 1 mL of the upper part of the suspension and measuring the absorbance ($\mathbf{A_t}$) at 600 nm. The percentage of auto-aggregation was calculated according to the formula:

$$\textit{Auto-aggregation \%} = 1-(A_t/A_0) \times 100$$

Where $\mathbf{A_t}$ represented the absorbance at time t = 2 h and $\mathbf{A_0}$ the absorbance at t = 0 h.

For co-aggregation, equal volumes (5 mL) of each cell suspension (x and y) were mixed (x + y) and vortexed for 30 sec. In the same time, control tubes containing 10 mL of each cell suspension (x or y) were prepared. The absorbance at 600 nm was measured after 2

h for each single suspension and then the mixture (Ax, A y and Ax+y). The percentage of co-aggregation was calculated using the formula:

$$\textit{Co-aggregation \%} = \frac{(Ax+Ay)/2-A(x+y)\times 100}{(Ax+Ay)/2}$$

Where x and y represented each of the two strains in the control tubes, and (x + y) the two strains in the mixture.

16. Novel antibacterial agents

The SAAP-148 peptide (amide-LKRVWKRVFKLLKRYWRQLKKPVR-acetyl; Mw 3970) was synthesized by 9H-fluorenylmethyloxycarbonyl (F-moc) chemistry on an automated peptide synthesizer (Synroll, MultiSyntech, Witten, Germany) as described previously **(Hiemstra *et al*., 1997; de Breij *et al*., 2018)**. The molecular mass of the peptide was confirmed by mass spectrometry (Voyager DE-Pro, PerSeptive Biosystems, Framingham, MA, USA) **(Nell *et al*., 2006)** and purity amounted >95%, as determined by reverse phase high-performance liquid chromatography (RP-HPLC) and detection at 214 nm **(Nell *et al*., 2006)**. Lyophilized peptide was stored at -20°C until use. A stock solution of 5.12 mM SAAP-148 was prepared in MilliQ water and stored in the refrigerator at 4°C. Halicin (SU 3327; Mw 261.3) was purchased from Tocris Bioscience (Bristol, UK). Prior to use in experiments, halicin was dissolved in 748 µL of dimethylsulfoxyde (DMSO; Honeywell Riedel-de-Haën, Seelze, Germany) and further diluted in PBS (140 mM, pH=7.4; Fresenius KABI, Zeist, the Netherlands) to a stock solution of 51.2 mM.

17. *In vitro* killing assay

From glycerol stock suspensions of *E. coli* (EC2), *K. pneumoniae* (KP1 and KP2) and *A. baumannii* (AB1), overnight cultures were prepared at 37 °C. Thereafter, the bacteria were cultured to the mid-log phase in TSB (Oxoid, Basingstoke, UK) for 2.5 h under continuous rotation (200 rpm), centrifuged at 3000 rpm for 10 min, and resuspended in PBS to a concentration of 5 × 10^6 CFU/mL. Subsequently, 30 µL of PBS containing SAAP-148 or halicin (final concentrations ranging from 0.8 to 102.4 µM) and 20% (*v*/*v*) bacterial suspension were mixed with either 50% (*v*/*v*) pure PBS; or 50% (*v*/*v*) pooled urine (for EC2, KP1 and KP2) or 50% (*v*/*v*) pooled human plasma (for AB1) in the wells of a polypropylene V-shape microplate (Greiner bio-one). After incubation for 4 h and 24 h at 37 °C (200 rpm),

the number of viable bacteria was assessed microbiologically. The results were expressed as lethal concentration (LC) 99.9, i.e., the lowest concentration of the compound that killed 99.9% of the *inoculum* **(de Breij *et al*., 2018)**.

18. Anti-biofilm assay

One-hundred microliters of a suspension of log-phase bacteria (approximately 10^7 CFU/mL in BHIB (Oxoid, Basingstoke, UK) were added to each well of a flat-bottom 96-well polystyrene microplate containing a sterile silicone elastomer disc (Ø = 4 mm), which mimics medical devices, like intravenous and urinary catheters, and incubated for 24 h at 37 °C. Thereafter, each disc was gently transferred in a sterile 96-well microplate, washed with PBS, and transferred again in a sterile flat-bottom 96-well polystyrene plate for exposure to increasing concentrations of SAAP-148 (in the range 6.4–102.4 µM) or halicin (in the range 6.4–204.8 µM) for 4 h and 24 h at 37 °C. Three discs were incubated with PBS as controls. In addition, three discs were exposed to 0.4% (*v*/*v*) DMSO as controls. The concentration of DMSO in the highest concentration of halicin that was tested served as a control for halicin. After the treatment, each disc was washed as described above; transferred to a sterile, flat-bottom, 96-well polystyrene microplate containing 100 µL of PBS; and sonicated for 10 min at 42 kHz. The number of surviving bacteria was determined microbiologically. The results were expressed as biofilm bactericidal concentrations (BBC99.9), i.e., the lowest concentrations of the agents resulting in a 99.9 % reduction in the bacterial counts in the biofilms **(de Breij *et al*., 2018)**.

19. Anti-persister assay

Seven-day mature biofilms were developed in flat-bottom, 96-well polypropylene plates using 100 µL of 1×10^7 CFU/mL bacteria in BHIB (Oxoid, Basingstoke, UK). The plates were sealed and incubated at 37 °C in a humidified atmosphere for 7 days. Thereafter, non-adherent bacteria were removed by two washes with PBS, and 100 µL of fresh BHIB containing 50 × MBC of ciprofloxacin (25 µg/mL; Sigma-Aldrich, St. Louis, MO, USA) for KP1, KP2, and EC1 and 50 × MBC of gentamycin (400 µg/mL; Sigma-Aldrich, St. Louis, MO, USA) for AB1 were carefully added to each well. The minimal bactericidal concentration (MBC) is the lowest concentration of the antibiotics that killed 99.9 % of the bacteria compared to the control **(Scheper *et al*., 2021).** The medium containing the antibiotics was refreshed daily for 3 days. After the treatment, the supernatant was removed; the biofilm was sonicated at 42 kHz for 10 min, and the resulting bacterial suspensions from

48 wells were pooled. A total volume of 10 µL of increasing doses of SAAP-148 or halicin (in the range 0.2–102.4 µM) was added to 90 µL of the pooled bacterial suspension and incubated for 4 h at 37 °C (200 rpm). As a control, bacteria were exposed to PBS without SAAP-148/halicin. Afterward, the number of viable bacteria was determined microbiologically. To exclude the possibility that persisters surviving the treatment were missed, the incubation on the agar plates was prolonged for up to 5 days **(de Breij *et al.*, 2018).**

20. Checkerboard assay for determination of SAAP-148 and halicin synergy

A checkerboard assay was used to determine possible synergism between SAAP-148 and halicin on bacteria in biofilms. In short, 24-h biofilms cultured on flat bottom polypropylene 96-wells plates containing a sterile silicone elastomer disc were washed two times with 100 µL of PBS and then exposed to combinations of increasing doses of SAAP-148 and halicin **(Appendix III)** for 24 h at 37°C. Thereafter, antimicrobial agents were gently removed; the plates were washed two times with PBS and sonicated for 10 min at 42 kHz. The number of surviving bacteria was determined microbiologically. The fractional biofilm eradication concentration index (ΣFBEC) was calculated according to the formula: $\Sigma FBEC = FBEC_A + FEBC_B$ **(Paduszynska *et al.*, 2020)**, where $FBEC_A$ = MBEC of compound A in the combination/MBEC of compound A, and $FBEC_B$=MBEC of compound B in the combination/MBEC of compound B. Synergy is defined as: $\Sigma FBEC \leq 0.5$, additive effect as $0.5 < \Sigma FBEC \leq 1$, indifference as $1 < \Sigma FBEC \leq 2$, and antagonism as $\Sigma FBEC > 2$.

21. Assay for bacterial Endotoxin neutralization capacities of SAAP-148 and halicin

A protocol developed in the laboratory of Department of Infectious diseases (Leiden university medical center) was used for the determination of the endotoxin neutralization capacities of SAAP-148 and halicin. For this, approximately 15 µL of UV-inactivated (for 20 min; ChemiDoc, Bio-Rad, Hercules, California, USA) bacteria (1×10^7 CFU/mL in PBS) were mixed with 15 µL of SAAP-148 or halicin at final concentrations ranging from 1 to 1000 nM in a 96-well polypropylene V-bottom microplate and incubated for 2 h at 37 °C. Thereafter, 120 µL of Roswell Park Memorial Institute medium (RPMI, Thermo Fisher, Paisley, UK) was added to each well to dilute the samples five times (5×). Next, 5 µL of this mixture was added to 195 µL of 5× diluted heparinized blood obtained from healthy donors, and the mixture was incubated for 20 h at 37 °C under 5% CO_2. Subsequently, the plates were spun for 5 min at 1200 rpm; the supernatants were aliquoted and stored at −20 °C until further

analysis. The level of the pro-inflammatory cytokine IL-12p40 in these supernatants was quantified using the BioLegend Elisa max deluxe set (BioLegend, San Diego, CA, USA) according to manufacturer's instructions, and the optical density (OD) at 450 nm was measured on a Spectramax i3X (Molecular Devices, Wokingham, UK).

22. Hemolysis assay for antimicrobial agents

The hemolytic activities of the peptide SAAP-148 and halicin were investigated. Briefly, freshly drawn citrate blood, from healthy volunteers, was washed three times with PBS by centrifugation at 1000 rpm for 10 min and then resuspended in PBS to prepare an erythrocyte suspension of 20% (*v*/*v*). Subsequently, 25 µL of PBS containing SAAP-148 or halicin (at final concentrations ranging from 12.8 to 204.8 µM) and 2% (*v*/*v*) RBC suspension were mixed with 50 µL of PBS or pooled human plasma in a 96-well polypropylene V-bottom plate (Greiner bio-one), shaken for 10 sec at 300 rpm, and incubated for 1 h at 37 °C. DMSO and 5% (*v*/*v*) Triton-X (Fluka Chemie, Buchs, Switzerland) were used as controls for 0% and 100% hemolysis, respectively. After centrifugation for 3 min at 1200 rpm, the supernatant was carefully transferred to new 96-well, polypropylene flat-bottom plates, and the hemoglobin release was determined by measuring the OD at 415 nm. The percentage of hemolysis was calculated as $(OD415_{sample} - OD415_{0\mu M})/(OD415_{TritonX} - OD415_{0\mu M}) \times 100\%$, where $OD415_{sample}$ is the optical density of the peptide/halicin, $OD415_{0\mu M}$ is the optical density of the negative control, and $OD415_{TritonX}$ is the optical density of the positive controls. Hemolysis ≤ 5% was considered as safe **(van Gent *et al.*, 2022)**.

23. Statistical analysis

Statistical analysis was performed using GraphPad Prism software version 6.0 (Graph Pad Software, San Diego, CA, USA). The pearson correlation was applied to study the relationship between the biofilm formation and virulence genes; biofilm formation and hydrophobicity; biofilm formation and auto-aggregation; and between biofilm formation and antibiotic sensitivity. The Kruskall-Wallis and Mann-Whitney U tests were performed to determine the significance of the differences between the values for peptide- and/or halicin-exposed and control samples. All data are presented as medians; $P \leq 0.05$ was considered statistically significant.

Results

1. Samples

The medical devices were coded "IVC" for intravenous catheter, "URC" for urinary catheter and "IAD" for intra-abdominal drain. From the 40 collected medical devices (28 IVCs, 8 URCs and 4 IADs), 20 of them were obtained from General Surgery department (with 65%, 20% and 15% of IVCs, IADs and URCs, respectively); 13 from Internal Medicine department, with a high rate of IVCs (92.3%) and 7.7% were URCs; and 6 from Resuscitation department, with 50% of IVCs and URCs respectively. Only 1 URC was collected from Infectious diseases department **(Figure 8, appendix IV)**.

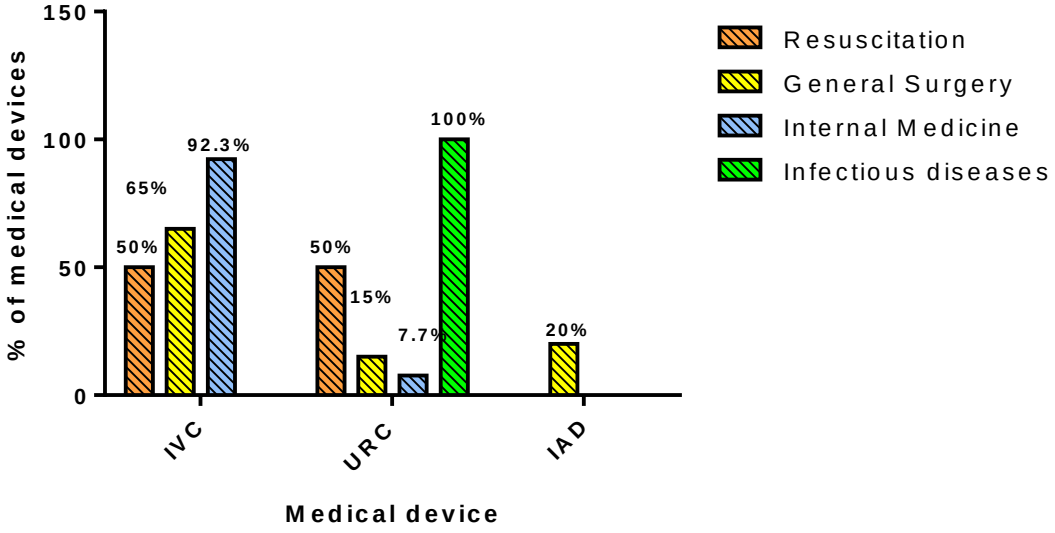


Figure 8. Distribution of the medical devices depending on the healthcare setting department
IVC: Intravenous catheter; **URC**: Urinary catheter; **IAD**: Intra-abdominal drain.

The collected medical devices belonged to 19 women and 20 men. Concerning IVCs, 53.57% (15/28) of these devices were collected from men and 46.43% (13/28) from women. However, the most URCs were collected from women (7/8; 87.5%). All IADs (4) were taken from men **(Figure 9, appendix IV)**. It should be mentioned that URC5 and IVC9 were collected from the same patient.

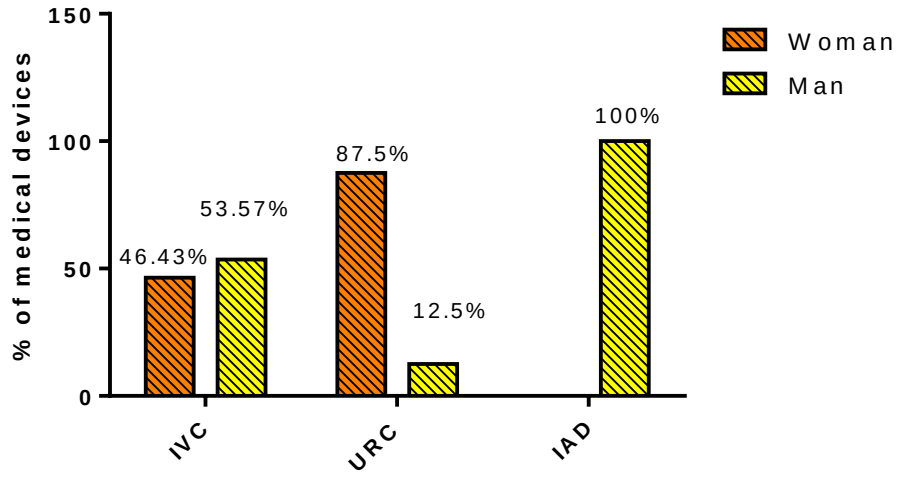


Figure 9. Distribution of the medical devices depending on the gender
IVC: Intravenous catheter; **URC:** Urinary catheter; **IAD:** Intra-abdominal drain

The age of the patients was ranged between 17 and 96 years old with an average age of 47.4 years old **(Table 11)**. The duration of medical device *in situ* was between 2 and 16 days with a large number of devices in the range of 3-7 days **(Figure 10, appendix IV)**. The 39 patient's presented different reasons for their hospitalization, including serious pathologies. Moreover, no local or general signs were observed in these patients wearing one of these medical devices **(Appendix IV)**.

Table 11. Distribution of the medical devices according to the patient's age

Age group (years)	Number of patients	Rate (%)
17-35	14	35.90
36-55	12	30.76
56-75	8	20.51
76-96	5	12.82

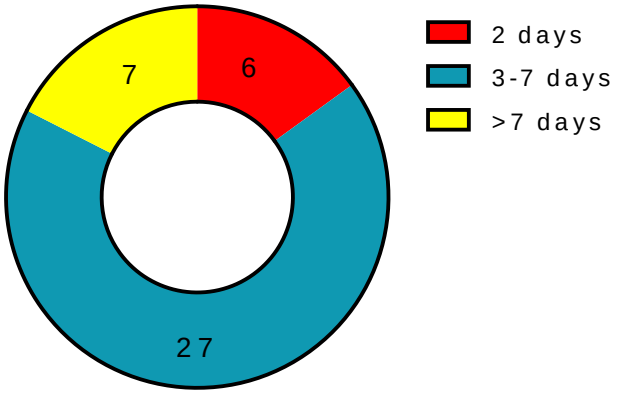


Figure 10. Distribution of the number of medical devices depending on the duration of the device *in situ*

2. Urine analysis

The urine samples, collected from catheterized patients, were coded US1-US8. The macroscopic appearance of the urine samples varied from slightly brownish, cloudy to highly cloudy urines. The pH of urines ranged from 4.65 to 7.13. The presence of leukocytes, red blood cells and epithelial cells was observed. The calcium oxalate crystals were found in two urine samples (US4, US6). The urine bacterial culture was positive in 75% (6/7) of urine samples on the day of sampling with a bacteriuria $\geq 10^5$ CFU/mL and $<10^4$ CFU/mL, in 4 and 2 samples respectively. No growth was observed in US1. The results of the urine samples analysis are resumed in the **table 12**. It should be mentioned that unfortunately the sample US5 was threw out by the medical staff before analysis.

Table 12. Urine samples analysis results

Urine code	Macroscopic appearance	pH	Microscopic appearance	Microorganisms Enumeration (CFU/mL)
US1	Slightly brownish urine	6.74	Leukocytes (12000/mL) Red blood cells (3500/ mL) Some epithelial cells	No growth
US2	Cloudy urine	4.88	Leukocytes (35000/ mL) Red blood cells (12000/ mL) Many epithelial cells Microorganisms	NA : 2.8×10^5 TSA : 1.7×10^5
US3	Cloudy urine	5.70	Leukocytes (20000/ mL) Red blood cells (5000/ mL) Microorganisms Some epithelial cells	NA : 2.1×10^2 TSA : 1.6×10^2
US4	Highly cloudy urine	7.13	Leukocytes (12000/ mL) Red blood cells (2000/ mL) Microorganisms Calcium oxalate crystals (+)	NA : 5.37×10^6 TSA : 1.2×10^7
US5			Not analysed	
US6	Cloudy urine	5.82	Leukocytes (25000/ mL) Red blood cells (6000/ mL) Microorganisms Many epithelial cells Calcium oxalate crystals (+)	NA : 7.8×10^6 TSA : 4.5×10^6
US7	Highly cloudy urine	4.65	Leukocytes (14000/ mL) Red blood cells (11000/ mL) Microorganisms Some epithelial cells	NA : 2.1×10^6 TSA : 8.1×10^5
US8	Cloudy urine	7.05	Leukocytes (10000/ mL) Red blood cells (1000/ mL) Microorganisms Some epithelial cells	NA : 5.4×10^3 TSA : 1.3×10^3

NA: Nutrient agar; **TSA:** Trypticase soy agar

3. Medical devices analysis

As mentioned in materials and methods section, 33/40 of the medical devices (insertion≤7 days) were subjected to bacteria enumeration and isolation. After enumeration on the different media, the results showed that 12/33 (36.4%) of the medical devices were colonized with a culture $\geq 10^3$ CFU/mL, 9 were IVCs and 3 were URCs; 15 medical devices were contaminated with less than 10^3 CFU/mL. However, no bacterial growth was observed in six IVCs (IVC13, IVC14, IVC21, IVC22, IVC25 and IVC28) **(Table 13, appendix V)**.

Table 13. Results of bacterial enumeration from the medical devices (insertion≤7 days)

	IVC (N=27)	**URC (N= 4)**	**IAD (N= 2)**
Infection (Culture ≥10^3 CFU/mL+ Symptoms)	0	0	0
Colonization (Culture ≥10^3 CFU/mL)	9	3	0
Contamination (Culture <10^3 CFU/mL)	12	1	2
Negative culture	6	0	0

IVC: Intravenous catheter; **URC**: Urinary catheter; **IAD**: Intra-abdominal drain

4. Phenotypic identification of the bacterial strains recovered from the medical devices

A total of 219 strains were isolated from the analysed medical devices (168 from IVCs, 32 from URCs and 19 from IADs). The phenotypic identification was possible, on the basis of cultural, morphological, biochemical and physiological characteristics. The results of the phenotypic identification are summarized below.

The strains were assigned to the species *Escherichia coli* (31 strains), *Klebsiella pneumoniae* (6 strains) and the genera *Acinetobacter* spp., (6 strains), *Staphylococcus* spp. (143 strains) and *Enterococcus* spp. (33 strains).

- The *E. coli* strains were recovered from EMB agar plates on the basis of their macroscopic aspect (Violet colonies or black colonies with a green metallic sheen; lactose positive) following incubation at 37°C and 44°C. They were characterized as Gram negative, rod-shaped or cocobacilli, facultative anaerobes, motiles, with catalase positive, indole positive, urease negative, VP negative, MR positive, citrate negative and nitrate reductase positive reactions.
- The *K. pneumoniae* strains were recovered from EMB agar plates (large, pink and mucoid colonies), with distinct characteristics: Gram negative, rod shaped, facultative anaerobes, non-motiles, with catalase-positive, indole negative, urease positive, VP positive, MR negative, citrate positive and nitrate reductase positive reactions**.**
- The *Acinetobacter* spp. strains were picked up from EMB agar plates (large, pink and mucoid colonies). They displayed characteristic traits: Gram negative, cocobacilli, strict aerobes, non-motiles, with catalase-positive, indole negative, urease negative,

VP negative, MR negative, citrate positive and nitrate reductase negative reactions.

- The *Staphylococcus* spp. isolates displayed these characteristics: Gram positive, cocci arranged in grape-like clusters, facultative anaerobes, non-motiles, with a catalase-positive reaction. These isolates were recovered from MSA. From the 143 isolates, 24 strains were coagulase positive, with golden yellow and creamy colonies on MSA. The remaining 119 strains were coagulase negative with pink and creamy colonies on MSA.
- The *Enterococcus* spp. isolates were picked up from BEA plates (small black colonies) with typical characteristics: Gram positive, cocci, facultative anaerobes, non-motiles, catalase-negative, able to grow under harsh conditions (pH=9.6, 6.5% [w/v] NaCl) and resist to thermal treatment (63°C/30 min).

5. Distribution of the isolated strains following the medical devices

Among the 168 isolated strains from IVCs, *Staphylococcus* spp. were the most frequently isolated species with a rate of 72 % (121/168) followed by *Enterobacteriaceae* (26/168; 15.5%), *Enterococcus* spp. (15/168; 8.9%) and *Acinetobacter* spp. (6/168; 3.6%). Same bacterial dominance was observed on IADs with 84.2 % (16/19) *Staphylococcus* spp. and 15.8 % (3/19) *Enterobacteriaceae*, with absence of *Enterococcus* spp. and *Acinetobacter* spp.. Regarding the URCs, *Enterococcus* spp. were the most predominant (56.3%; 18/32), followed by *Enterobacteriaceae* (25%; 8/32), and *Staphylococcus* spp. (18.8%; 6/32) **(Figure 11)**.

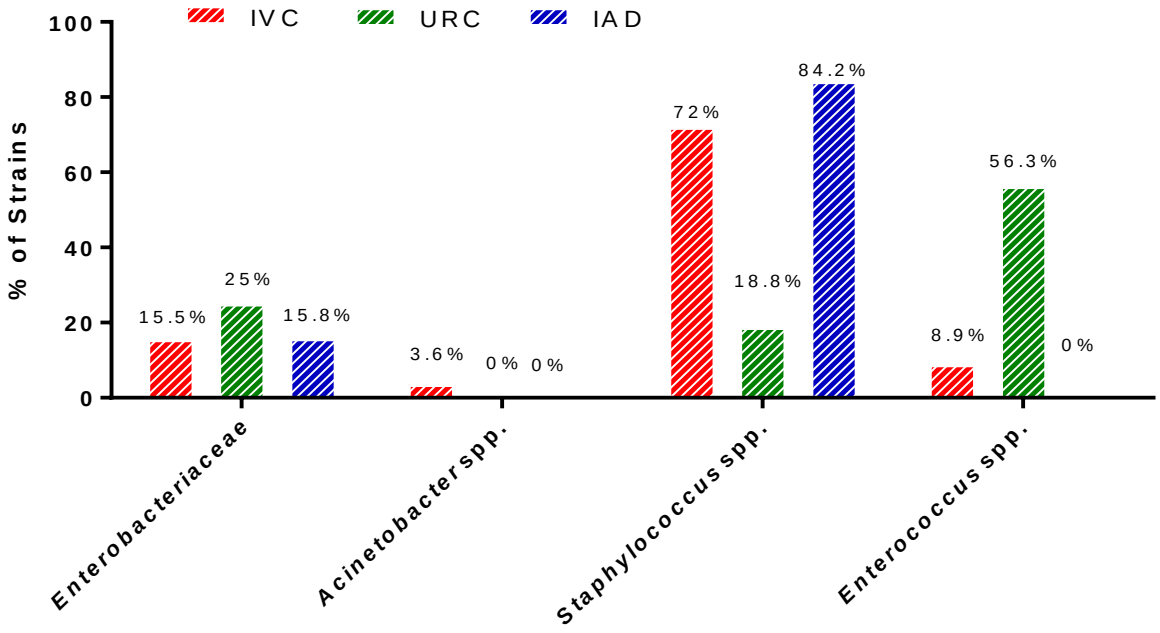


Figure 11. Distribution (%) of the isolates following the medical device

6. Molecular identification of the bacterial isolates colonizing the medical devices

Among the 219 isolates, 104 strains were identified by 16S rDNA amplification thereby sequence analysis and BLAST. A total of 33/219 (15.07%) strains were identified as *Enterococcus faecalis* (EF1—EF33), 31/219 (14.16%) as *Escherichia coli* (EC1—EC31), 24/219 (10.96%) as *Staphylococcus aureus* (ST1-ST3; ST8-ST11; ST12-ST21; ST27; ST34-ST35; ST40 and ST42-ST44), 6/219 (2.74%) as *Klebsiella pneumoniae* (KP1-KP6), 5/219 (2.29%) as *Acinetobacter baumannii* (AB1-AB5), 3/219 (1.37%) as *Staphylococcus haemolyticus* (ST31, ST32, ST33), 1/219 (0.46%) as *Staphylococcus cohnii* (ST4) and 1/219 (0.46%) as *Acinetobacter lwoffii* (AL). The remaining strains (115; 52.51%) were identified as other Coagulase negative Staphylococci **(Figure 12, Appendix VI)**.

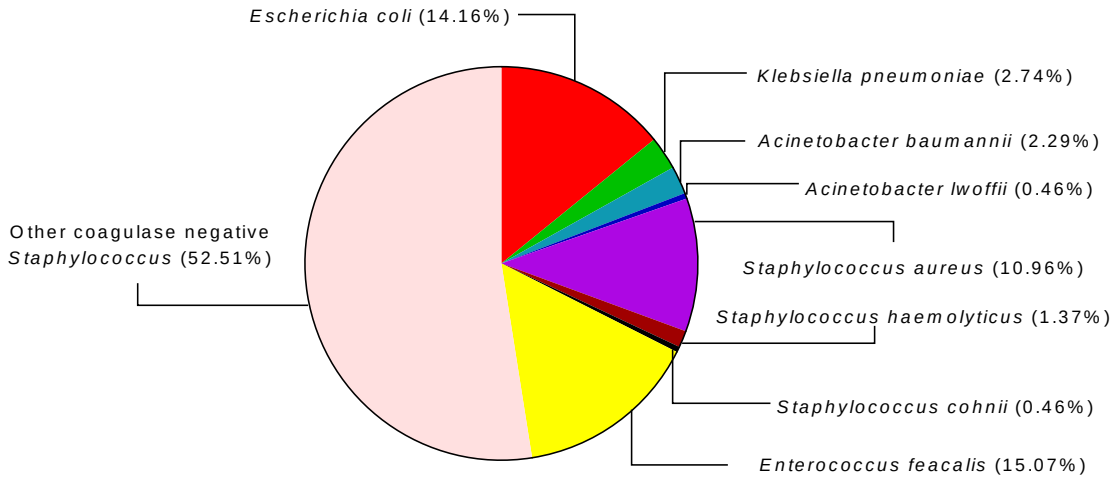


Figure 12. Distribution of the isolates recovered from the medical devices.

7. Diffusion of bacteria forming biofilm from colonized medical devices

To study the diffusion of bacteria forming biofilms on the medical devices left more than 7 days (7-16 days) *in situ*, 1 intravenous catheter (IVC9), 4 urinary catheters (URC1, URC2, URC4 and URC5) and 2 intra-abdominal drains (IAD2 and IAD4) were included. Discs and pieces were cut and cultured on Columbia and TSA blood agars. After 24 h of incubation, a dense bacterial growth was observed around two medical devices (URC4 and IAD2). However, a bacterial growth around URC5 was observed after 48 h and no growth was observed from the devices URC1, URC2, IVC9 and IAD4 even after 72 h of incubation. The **figure 13** showed the bacterial growth from the devices URC4 and IAD2. Greenish hemolytic zones were observed, indicating the α-hemolytic character of the bacterial strains colonizing these devices.

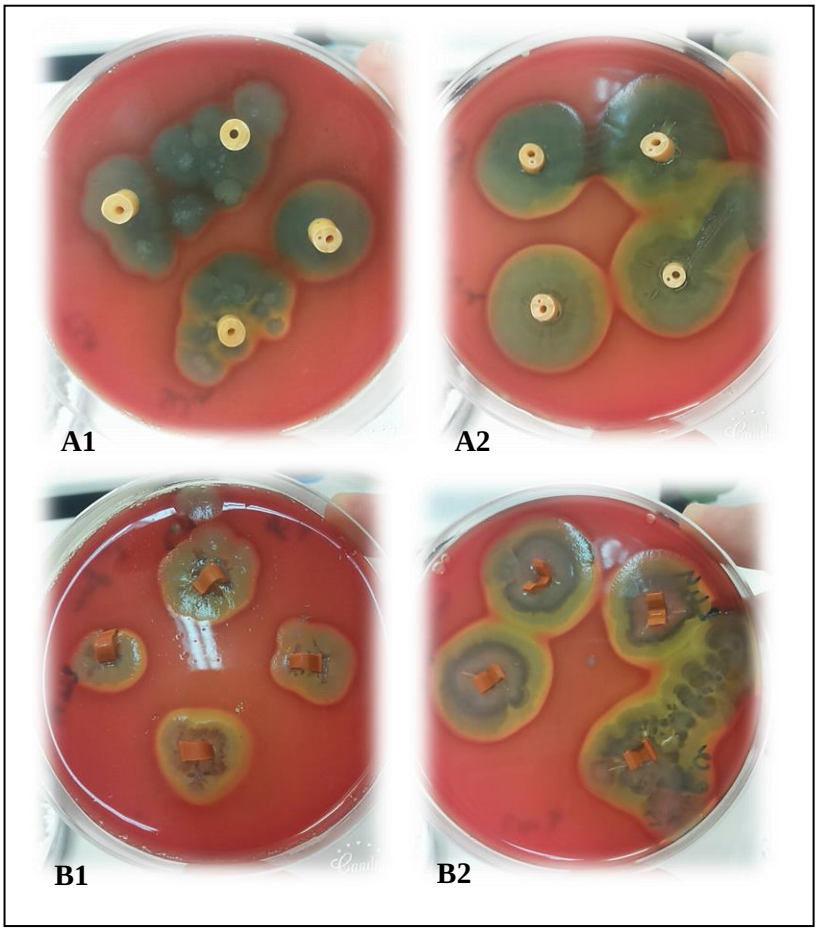


Figure 13. Photographies of bacterial growth obtained from two medical devices discs cultured on blood agar plates after 24 h of incubation. **(A1)** URC4 on TSA blood agar; **(A2)** URC4 on Columbia blood agar; **(B1)** IAD2 on TSA blood agar; **(B2)** IAD2 on Columbia blood agar

8. Bacterial biofilm formation

To characterize the pathogenic bacteria originating from the medical devices, their ability to form biofilms was assessed and classified into: strongly biofilm producer, moderately biofilm producer, weakly biofilm producer and non biofilm producer **(Figure 14)**. Results are expressed as mean and compared to $OD_{630\ nm}$ for negative control (OD_{630nm}) amounted to 0.032±0.01. Results revealed that biofilm formation on polystyrene microplates varied widely among the strains showing that 85.84% of the strains were significantly (p<0.05) strongly adherent, and consequently strong biofilm producers, against a rate of 3.65% for non adherent (non biofilm-producer) strains **(Table 14)**.

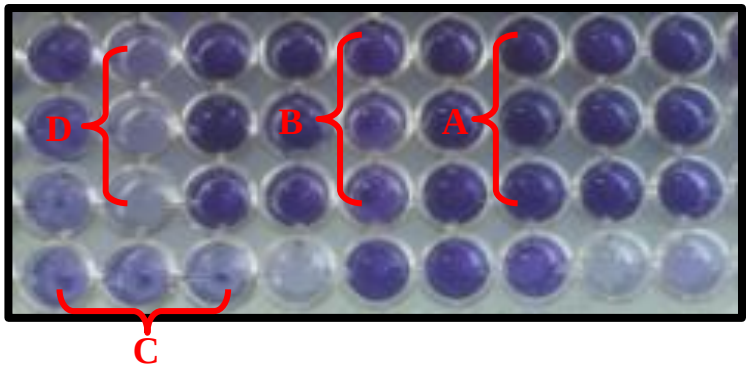


Figure 14. Photography of biofilms in 96-well polystyrene microplate after staining with crystal violet. **(A)** Strong adherent strain, **(B)** Moderate adherent strain, **(C)** Weak adherent strain, **(D)** Non-adherent strain

Table 14. Classification of the isolated strains based on their adhesion potential on polystyrene microplates.

	Biofilm formation (TCP assay)			
	OD > 0.12	0.06 < OD ≤0.12	0.03 < OD ≤0.06	OD ≤0.03
	Strong (%)	Moderate (%)	Weak (%)	Negative (%)
% of strains	85.4	5.94	5.02	3.65
E. coli	38.71	19.35	22.58	19.35
K. pneumonia	83.33	16.67	0	0
Acinetobacter spp.	33.33	16.67	33.33	16.67
Staphylococcus spp.	94.41	3.50	1.39	0.7
En. faecalis	100	0	0	0

0.03= OD control

8.1. *Enterobacteriacae*

In *Enterobacteriacae* strains, the biofilm formation on polystyrene microplates varied largely among the *E. coli* strains (12/31 were strong producers) **(Table 14, Figure 14)**, while all the *K. pneumoniae* strains formed significant (p<0.05) biofilms with 5/6 of them being strong producers **(Table 14, Figure 15)**. The results of TCP assay are summarized in the **appendix VII.**

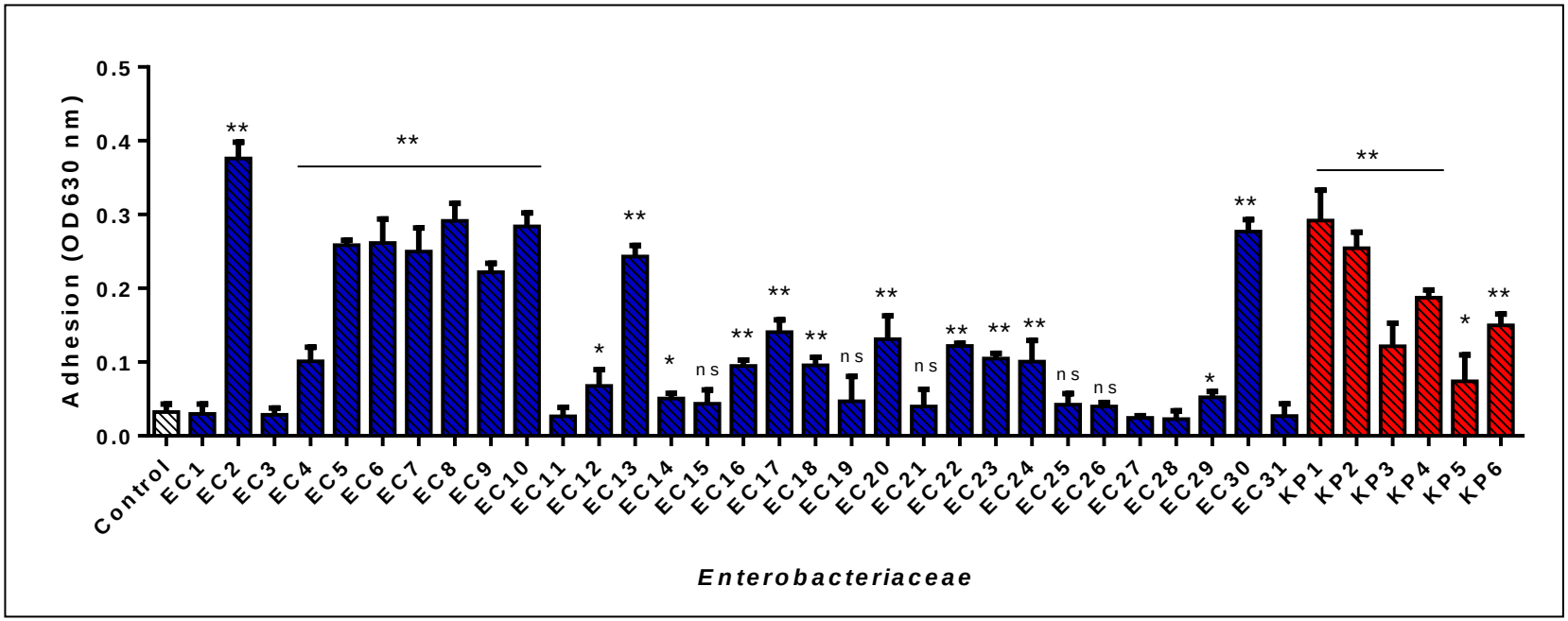


Figure 15. Biofilm formation ability of *Enterobacteriaceae* isolated from medical devices on 96-well polystyrene microplate. **EC:** *E. coli*; **KP:** *K. pneumoniae*; **White bar:** Negative control. n=3 independent experiments each in triplicate. Biofilm is formed if OD_{sample} is significantly higher ($^{*}p<0.05$, $^{**}p<0.01$, Mann Whitney u test) than $OD_{control}$. **ns:** not significantly different.

8.2. *Acinetobacter* spp.

Almost all *A. boumannii* strains were biofilm producers (except the strain AB5) with 2/5 being strong producers. Similarly, *A. lwoffii* was also a strong producer strain **(Table 14, Figure 16)**. The results of TCP assay are summarized in the **appendix VII.**

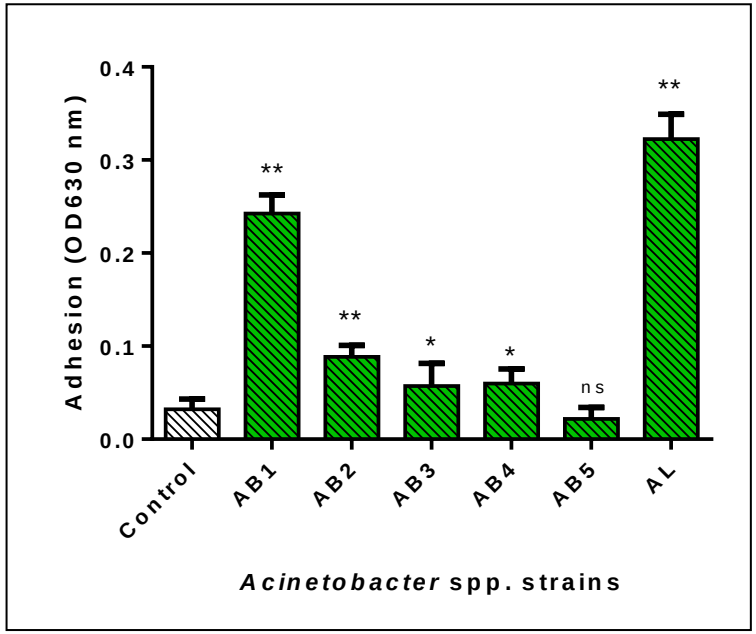


Figure 16. Biofilm formation ability of *Acinetobacter* spp. isolated from medical devices on 96-well polystyrene microplate. **AB:** *A. baumannii*; **AL:** *A. lwoffii*; **White bar:** Negative control. n=3 independent experiments each in triplicate. Biofilm is formed if OD_{sample} is significantly higher (****p*<0.05**, *****p*<0.01**, Mann Whitney u test) than $OD_{control}$. **ns:** not significantly different.

8.3. *Enterococcus faecalis*

For *En. faecalis*, all strains were significantly (p<0.01) strong biofilm producers (100%) **(Table 14, Figure 17)**. The results of TCP assay are summarized in the **appendix VII.**

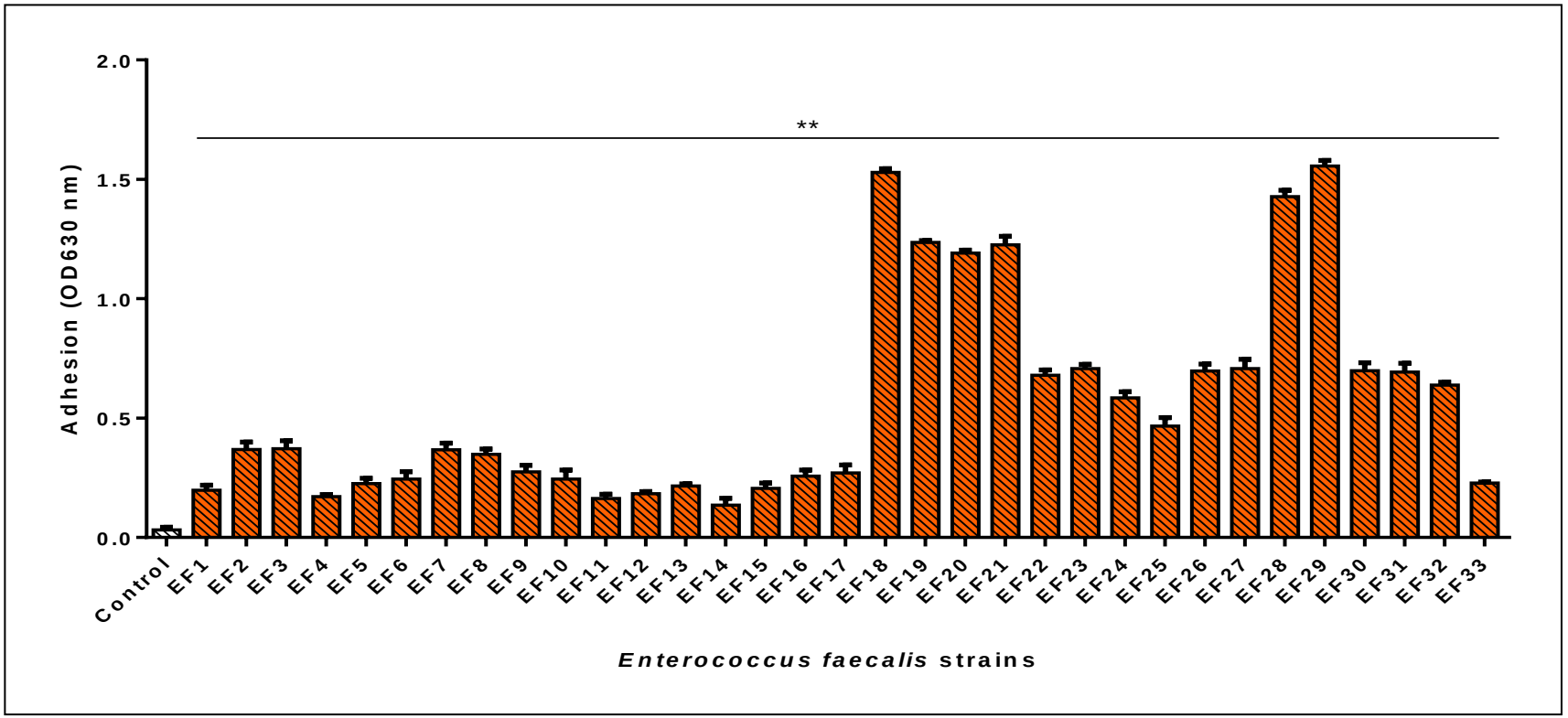


Figure 17. Biofilm formation ability of *Enterococcus faecalis* strains isolated from medical devices on 96-well polystyrene microplate. **White bar:** Negative control. n=3 independent experiments each in triplicate. Biofilm is formed if OD_{sample} is significantly higher (**p<0.01**, Mann Whitney u test) than $OD_{control}$.

8.4. *Staphylococcus* spp.

The ability to form biofilm in *Staphylococcus* spp. strains was variable with a high rate of strong producer strains (94.41%) **(Table 14, Figures 18 A-B)**. The results of TCP assay are summarized in the **appendix VII.** For *S. aureus*, all strains were significantly (p<0.05) strong biofilm producers (100%) **(Figure 18A)**.

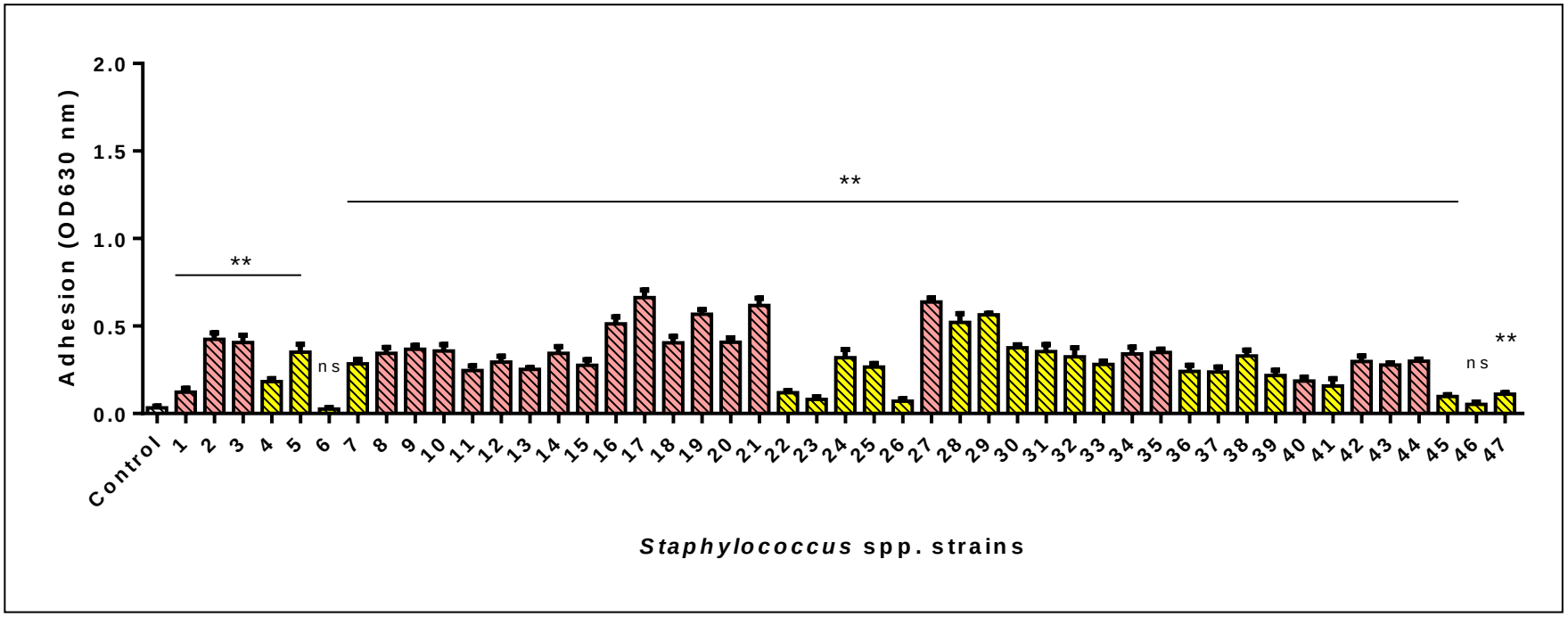


Figure 18A. Biofilm formation ability of some *Staphylococcus* spp. strains (ST1-ST47) isolated from medical devices on 96-well polystyrene microplate. **Pink bar**: *Staphylococcus aureus*. **Yellow bar**: Coagulase negative *Staphylococcus*. **White bar:** Negative control. n=3 independent experiments each in triplicate. Biofilm is formed if OD_{sample} is significantly higher (**$^{**}p$<0.01**, Mann Whitney u test) than $OD_{control}$. **ns:** not significantly different.

In coagulase negative *Staphylococcus*, the biofilm formation on polystyrene microplates was significantly (p<0.01) strong for 111/119 strains and moderate for 5/111 strains **(Table 14, Figures 18 A-B-C)**, while ST6, ST46 and ST70 strains were not significantly (p>0.05) producers of biofilms **(Table 14, Figures 18 A-B)**.

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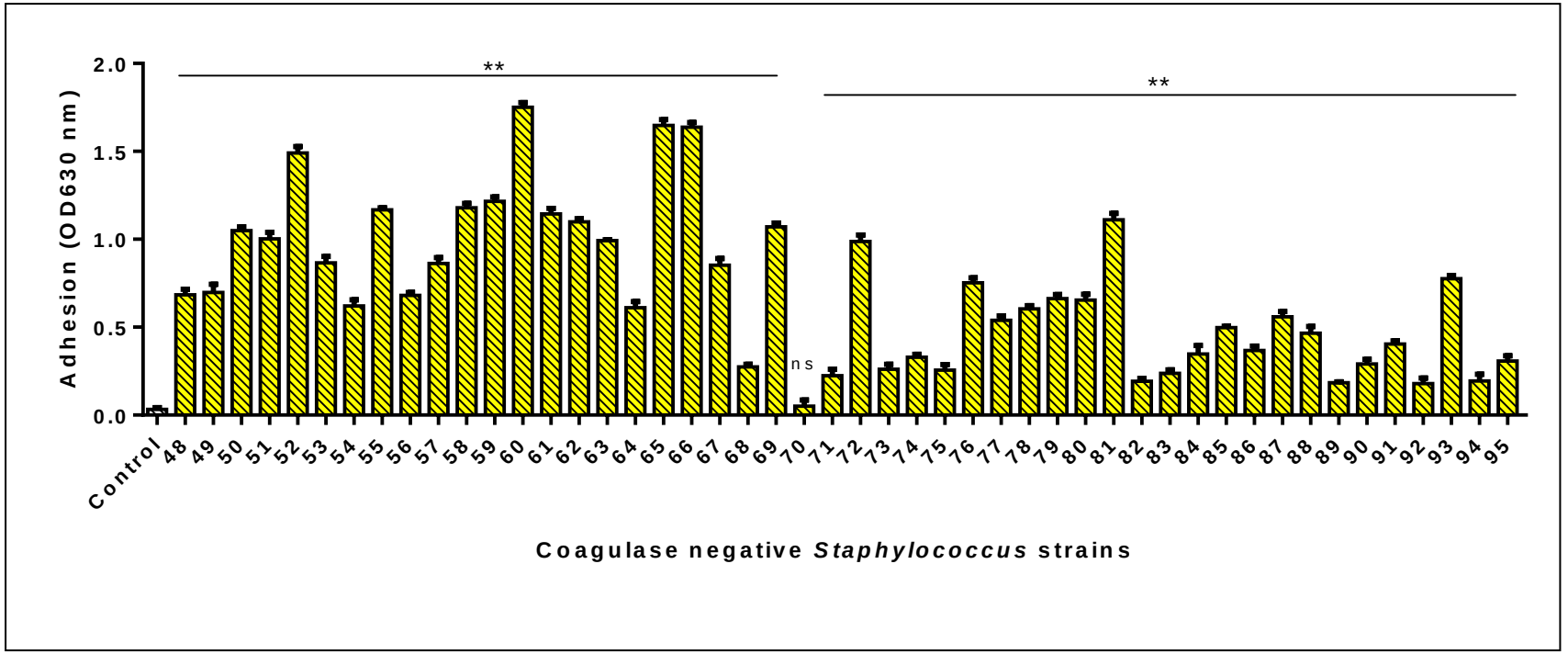


Figure 18B. Biofilm formation ability of the Coagulase negative *Staphylococcus* strains (ST48-ST95) isolated from medical devices on 96-well polystyrene microplate. **White bar:** Negative control. n=3 independent experiments each in triplicate. Biofilm is formed if OD_{sample} is significantly higher (**p<0.01**, Mann Whitney u test) than $OD_{control}$. **ns:** not significantly different .

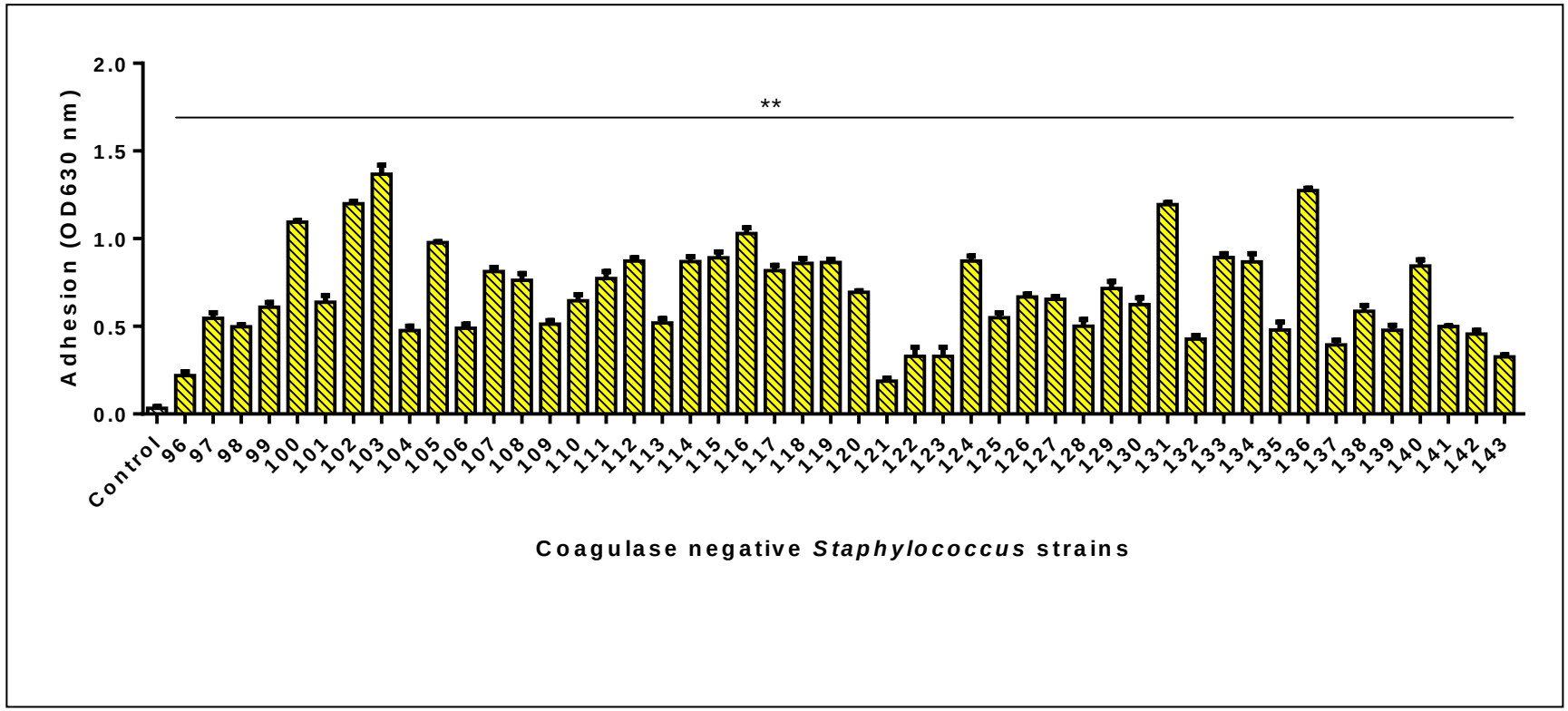


Figure 18C. Biofilm formation ability of the rest of Coagulase negative *Staphylococcus* strains (ST96-ST143) isolated from medical devices on 96-well polystyrene microplate. **White bar:** Negative control. n=3 independent experiments each in triplicate. Biofilm is formed if OD_{sample} is significantly higher (*****p*<0.01**, Mann Whitney u test) than $OD_{control}$.

9. Slime production

Slime production was determined using the Congo red agar (CRA) method. The appearance of the colonies on CRA medium is showed in the **figure 19**. The results showed that only 10% of isolated strains produced slime using this assay **(Figure 20a)** with a high rate of *Staphylococcus* spp. strains. Indeed, 22/143 (15.38%) of *Staphylococcus* spp. strains were strong slime producers of which 15/22 (68.18%) were *S. aureus* and 7/22 (31.81%) were Coagulase negative Staphylococci. However, 70/143 (48.95%) of isolated strains were weak slime producers and 51/143 (35.66%) were negative slime producers **(Figure 20b)**. Interestingly, the production of slime was negative for *Enterobacteriaceae, Acinetobacter* spp. and *Enterococcus* spp.. The results of Congo red agar (CRA) method as summarized in **appendix VII.**

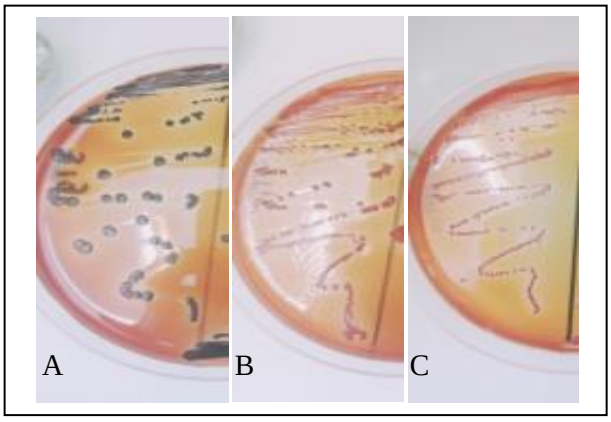


Figure 19. Appearance of colonies on CRA medium. Strong slime-producing strain **(A)**, weak slime-producing strain **(B)**, Non-slime-producing strain **(C)**.

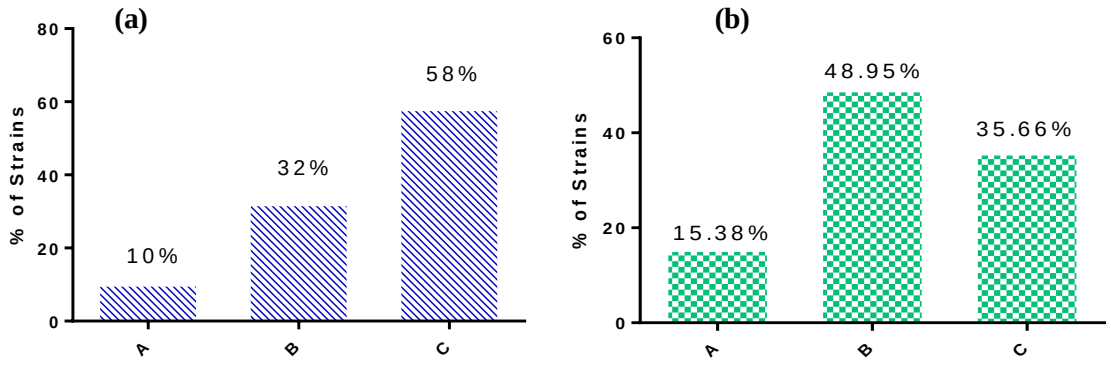


Figure 20. (a) Proportion of slim-producing strains detected by the CRA method. **(b)** Proportion of slim-producing strains in *Staphylococcus* spp. detected by the CRA method Strong slime-producing strains **(A)**, weak slime-producing strains **(B)**, Non-slime-producing strains **(C)**.

10. Virulence genes and antibiotic resistance profiles of the strains colonizing the medical devices

To further characterize these colonizing medical devices strains, the presence of virulence genes, especially those involved in adhesion and biofilm formation, was investigated by specific PCR. The virulence genes profile of the strains is presented in **tables 15 (A-E)**. Similarly, their antibiotic resistance profile was determined and presented in the same tables.

10.1. *Enterobacteriaceae*

All of the 31 *E. coli* (EC1-EC31) strains harboured the *fim*H gene, 30/31 strains the *csg*A gene and 15 out of the 31 strains (48.39%) the *hly*F gene. Virtually all *E. coli* strains were sensitive to almost all antibiotics tested except for nalidixic acid (77.42%), tetracycline (38.71%), amoxicillin/clavulanic acid (22.58%) and cotrimoxazole (9.68%) **(Table 15A, appendix VIII)**. Similarly, all of the 6 *K. pneumoniae* (KP1-KP6) strains harboured *mrk*D, *fim*H-1, *ycf*M and *ecp*A genes **(Table 15A)**. *K. pneumoniae* strains were resistant to β-lactams (except cefoxitin) and two out of six strains were resistant to gentamicin. Furthermore, a synergistic image was observed in all *K. pneumoniae* strains except KP1 indicating the probable production of an ESBL in these strains **(Figure 21, appendix VIII)**.

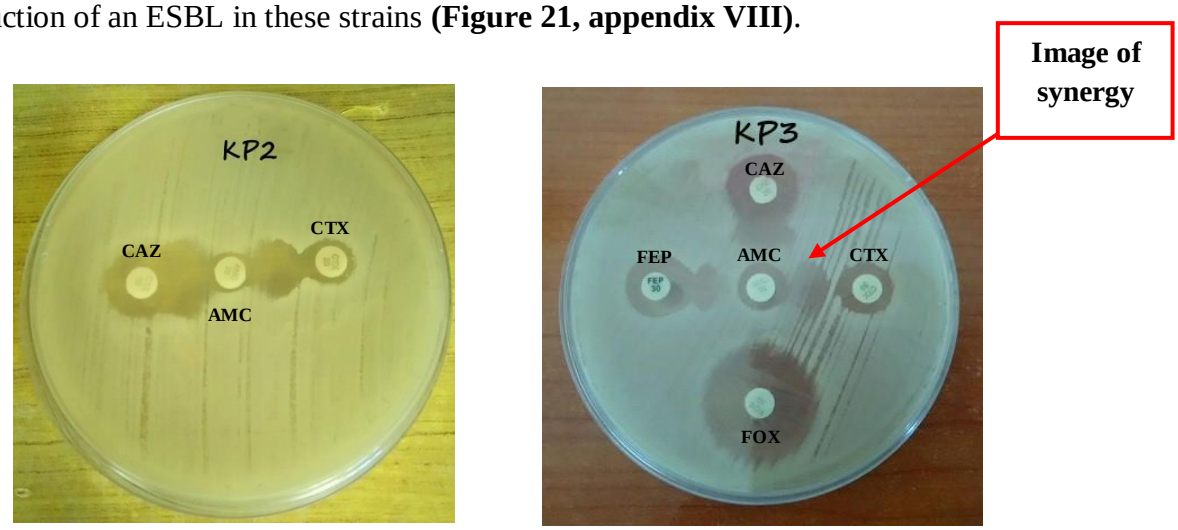


Figure 21. Photography of an ESBL-producing KP2 and KP3 strains after Double Disc Synergy Test (DDST). **AMC:** Amoxicillin+clavulanic acid, **CTX:** Cefotaxime, **CAZ:** Ceftazidime, **FEP:** Cefepim, **FOX:** Cefoxitin.

Table 15A. Virulence and antibiotic resistance profile of *Enterobacteriaceae* strains (EC1-EC31; KP1-KP6) isolated from medical devices. Only intermediate/resistance are indicated. Tested antibiotics: **AMC:** Amoxicillin+clavulanic acid, **CTX:** Cefotaxime, **CAZ:** Ceftazidime, **FOX:** Cefoxitin**,** **TET:** Tetracycline, **CIP:** Ciprofloxacin, **GEN:** Gentamicin, **IMP:** Imipinem, **FEP:** Cefepim, **NA:** Nalidixic acid, **CO-TRI:** Cotrimoxazole. **EC:** *Escherichia coli*, **KP:** *Klebsiella pneumoniae.*

Strain	Virulence profile	Resistance profile	Strain	Virulence profile	Resistance profile
EC1	*fimH+*, *hlyF+*, *csgA+*	TET^R, NA^R	**EC26**	*fimH+*, *hlyF+*, *csgA+*	AMC^R, NA^R
EC2	*fimH+*, *hlyF-*, *csgA+*	TET^R, GEN^I,NA^R	**EC27**	*fimH+*, *hlyF+*, *csgA+*	TET^R, NA^R
EC3	*fimH+*, *hlyF-*, *csgA+*	*Sensitive*	**EC28**	*fimH+*, *hlyF-*, *csgA+*	AMC^R, CO-TRI^R
EC4	*fimH+*, *hlyF-*, *csgA+*	NA^R	**EC29**	*fimH+*, *hlyF-*, *csgA-*	*Sensitive*
EC5	*fimH+*, *hlyF+*, *csgA+*	CIP^I, NA^R	**EC30**	*fimH+*, *hlyF-*, *csgA+*	CIP^I, NA^R
EC6	*fimH+*, *hlyF+*, *csgA+*	NA^R	**EC31**	*fimH+*, *hlyF-*, *csgA+*	TET^R, NA^R
EC7	*fimH+*, *hlyF-*, *csgA+*	NA^R	**KP1**	*mrkD+*, *fimH+*, *ycfM+*, *ecpA+*	AMC^R, FEP^R, TET^R, CIP^I, GEN^R
EC8	*fimH+*, *hlyF+*, *csgA+*	TET^I, NA^R	**KP2**	*mrkD+*, *fimH+*, *ycfM+*, *ecpA+*	AMC^R, CTX^R, CAZ^R, FEP^R, TET^R, GEN^I
EC9	*fimH+*, *hlyF+*, *csgA+*	TET^R, NA^R	**KP3**	*mrkD+*, *fimH+*, *ycfM+*, *ecpA+*	AMC^R, CTX^R, CAZ^R,FEP^R, TET^R, GEN^R
EC10	*fimH+*, *hlyF-*, *csgA+*	AMC^R, NA^R	**KP4**	*mrkD+*, *fimH+*, *ycfM+*, *ecpA+*	AMC^R, CTX^R, CAZ^R, FEP^R, TET^R
EC11	*fimH+*, *hlyF-*, *csgA+*	AMC^R, CO-TRI^R	**KP5**	*mrkD+*, *fimH+*, *ycfM+*, *ecpA+*	AMC^R, CTX^R, CAZ^R, FEP^R, TET^R, CIP^I, GEN^I
EC12	*fimH+*, *hlyF-*, *csgA+*	NA^R	**KP6**	*mrkD+*, *fimH+*, *ycfM+*, *ecpA+*	AMC^R, CTX^R, CAZ^R,FEP^R
EC13	*fimH+*, *hlyF-*, *csgA+*	CIP^I			
EC14	*fimH+*, *hlyF-*, *csgA+*	AMC^R, CO-TRI^R			
EC15	*fimH+*, *hlyF+*, *csgA+*	AMC^R, TET^R			
EC16	*fimH+*, *hlyF-*, *csgA+*	TET^R, NA^R			
EC17	*fimH+*, *hlyF-*, *csgA+*	TET^R, NA^R			
EC18	*fimH+*, *hlyF+*, *csgA+*	CIP^I, NA^R			
EC19	*fimH+*, *hlyF-*, *csgA+*	TET^R, NA^R			
EC20	*fimH+*, *hlyF+*, *csgA+*	CIP^I, NA^R			
EC21	*fimH+*, *hlyF+*, *csgA+*	TET^R, NA^R			
EC22	*fimH+*, *hlyF+*, *csgA+*	AMC^R, NA^R			
EC23	*fimH+*, *hlyF+*, *csgA+*	CIP^I, NA^R			
EC24	*fimH+*, *hlyF+*, *csgA+*	TET^R, NA^R			
EC25	*fimH+*, *hlyF+*, *csgA+*	TET^R, NA^R			

10.2. *A. baumannii*

All of the 5 *A. baumannii* (AB) strains exhibited the *omp*A and *csu*E genes, whereas four out of five strains exhibited the *bap* gene **(Table 15B, appendix VIII)**. *Acinetobacter* strains exhibited high levels of resistance to all antibiotics, except to gentamicin.

Table 15B. Virulence and antibiotic resistance profile of *Acinetobacter* spp. strains isolated from medical devices. Tested antibiotics: **CTX:** Cefotaxime, **CAZ:** Ceftazidime, **TET:** Tetracycline, **CIP:** Ciprofloxacin, **GEN:** Gentamicin, **IMP:** Imipinem, **FEP:** Cefepim, **CO-TRI:** Cotrimoxazole. **AB:** *Acinetobacter baumannii*, **AL:** *Acinetobacter lwoffii*.

Strain	Virulence profile	Resistance profile
AB1	*csuE+, ompA+,bap+*	CTX^R,CAZ^R, FEP^R,IPM^R,TET^R, CIP^R, CO-TRI^R
AB2	*csuE+, ompA+,bap+*	CTX^R,CAZ^R, FEP^R, IPM^R,TET^R, CIP^R, CO-TRI^R
AB3	*csuE+, ompA+,bap+*	CTX^R,CAZ^R, FEP^R, IPM^R,TET^R, CIP^R, CO-TRI^R
AL	*csuE-, ompA+,bap-*	FEP^R, IPM^R, TET^R, CIP^R, CO-TRI^R
AB4	*csuE+, ompA+,bap-*	CTX^R,CAZ^R, FEP^R, IPM^R,TET^R, CIP^R,CO-TRI^R
AB5	*csuE+, ompA+,bap+*	CTX^R,CAZ^R, FEP^R, IPM^R,TET^R, CIP^R, CO-TRI^R

10.3. *En. faecalis*

Among *En. faecalis* strains, 31/33 (93.93%) and 29/33 (87.88%) strains harboured *asa*1 and *esp* genes, respectively. Only seven strains harboured *gel*E gene **(Table 16C)**. Similarly, 87.88% (29/33), 81.82% (27/33) and 81.82% (27/33) *En. feacalis* strains were resistant to tetracycline, ciprofloxacin and erythromycin respectively. Only 15.15% (5/33) strains were resistant to rifampicin. In addition, 32/33 strains and 1/33 strains were intermediate to cotrimoxazole (96.97%) and imipinem (3.03%). Interestingly, all the strains were sensitive to vancomycin (MIC< 4 µg/mL) **(Table 15C, appendix VIII)**.

Table 15C. Virulence and antibiotic resistance profile of *Enterococcus faecalis* strains (EF1-EF28) isolated from medical devices. Only intermediate/resistance indicated. Tested antibiotics: **TET:** Tetracycline, **CIP:** Ciprofloxacin, **ERY:** Erythromycin, **IMP:** Imipinem, **CO-TRI:** Cotrimoxazole, **RIF:** Rifampicin. **Vancomycin resistance profile:** An E- test was assessed for the strains.

Strain	Virulence profile	Resistance profile	Strain	Virulence profile	Resistance profile
EF1	*esp-*, *gelE+*, *asa1+*	CIP^{I}, ERY^{I}, $CO\text{-}TRI^{I}$, RIF^{R}	**EF26**	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$, RIF^{I}
EF2	*esp-*, *gelE+*, *asa1+*	CIP^{I}, ERY^{I}, $CO\text{-}TRI^{I}$, RIF^{I}	**EF27**	*esp+*, *gelE+*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{R}$, RIF^{R}
EF3	*esp-*, *gelE+*, *asa1+*	CIP^{I}, ERY^{I}, $CO\text{-}TRI^{I}$	**EF28**	*esp+*, *gelE-*, *asa1-*	TET^{R}, CIP^{R}, ERY^{R}, IMP^{I}, $CO\text{-}TRI^{I}$
EF4	*esp-*, *gelE+*, *asa1+*	CIP^{I}, ERY^{I}, $CO\text{-}TRI^{I}$, RIF^{R}	**EF29**	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$, RIF^{I}
EF5	*esp+*, *gelE+*, *asa1-*	TET^{R}, CIP^{I}, ERY^{I}, $CO\text{-}TRI^{I}$	**EF30**	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$
EF6	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$	**EF31**	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$, RIF^{R}
EF7	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$	**EF32**	*esp+*, *gelE-*, *asa1+*	TET^{R}, $CO\text{-}TRI^{I}$, RIF^{I}
EF8	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$, RIF^{I}	**EF33**	*esp+*, *gelE+*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$, RIF^{I}
EF9	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$, RIF^{I}			
EF10	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$			
EF11	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$			
EF12	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$, RIF^{I}			
EF13	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$			
EF14	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$, RIF^{I}			
EF15	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$, RIF^{I}			
EF16	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$			
EF17	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$			
EF18	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$			
EF19	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$			
EF20	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$			
EF21	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$			
EF22	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$			
EF23	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$			
EF24	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$			
EF25	*esp+*, *gelE-*, *asa1+*	TET^{R}, CIP^{R}, ERY^{R}, $CO\text{-}TRI^{I}$, RIF^{R}			

10.4. *Staphylococcus* spp.

For *S. aureus* (SA), all strains harboured *fnbp*A, *clf*A and *hlb* genes, 23/24 (95.83) and 20/24 (83.33%) possess the *ica*D and *ica*A genes, respectively; whereas none *S. aureus* strain harboured *sas*G gene. Among the 23 coagulase-negative *Staphylococcus* strains, eight strains (34.78%) exhibited the *ica*A gene, 6/23 were *fnbpA*+ and 5/23 were *hlb*+. However, none strain harboured *ica*D, *embp* and *aap* genes **(Tables 15 D-E)**. Among *S. aureus* strains, the majority (20/24; 83.33%) of them were sensitive to all antibiotics except ST8 strain which was resistant to four antibiotics **(Table 15D, appendix VIII)**. Likewise, 44/119 (36.97%) of coagulase negative *Staphylococcus* strains were also susceptible to all antibiotics. However, these strains were resistant to erythromycin (55/119; 46.22%), tobramycin (51/119; 42.86%), cefoxitin (39/119; 32.77%), ciprofloxacin (38/119; 31.93%), gentamicin (37/119; 31.09%), cotrimoxazole (26/119; 21.85%), rifampicin (9/119; 7.56%) and tetracycline (8/119; 6.72%) **(Tables 15 D-E, appendix VIII).**

Table 15D. Virulence and antibiotic resistance profile of *Staphylococcus* spp. strains (ST1-ST47) isolated from medical devices. Only intermediate/resistance indicated. Tested antibiotics: **FOX:** Cefoxitin, **ERY:** Erythromycin, **GEN:** Gentamicin, **TET:** Tetracycline, **CIP:** Ciprofloxacin, **CFT:** Ceftaroline (Only *S. aureus*), **RIF:** Rifampicin, **TOB:** Tobramycin, **CO-TRI:** Cotrimoxazole.

Strain	Virulence profile	Resistance profile	Strain	Virulence profile	Resistance profile
ST1	*icaA-, icaD+, fnbpA+, clfA+, sasG-, hlb+*	*Sensitive*	**ST25**	*icaA+, icaD-, fnbpA+, embp-, aap-, hlb+*	ERY^R, TOB^R, $CO\text{-}TRI^R$
ST2	*icaA-, icaD+, fnbpA+, clfA+, sasG-, hlb+*	*Sensitive*	**ST26**	*icaA+, icaD-, fnbpA-, embp-, aap-, hlb-*	ERY^R
ST3	*icaA-, icaD+,fnbpA+, clfA+, sasG-, hlb+*	*Sensitive*	**ST27**	*icaA+, icaD+,fnbpA+, clfA+, sasG-, hlb+*	*Sensitive*
ST4	*icaA+, icaD-, fnbpA-, embp-, aap-, hlb-*	ERY^R, TET^R	**ST28**	*icaA-, icaD-, fnbpA-, embp-, aap-, hlb-*	ERY^R, $CO\text{-}TRI^I$
ST5	*icaA-, icaD-, fnbpA-, embp-, aap-, hlb-*	ERY^R, TET^R	**ST29**	*icaA-, icaD-, fnbpA-, embp-, aap-, hlb-*	TET^I
ST6	*icaA-, icaD-, fnbpA-, embp-, aap-, hlb-*	ERY^R, TET^R	**ST30**	*icaA-, icaD-, fnbpA-, embp-, aap-, hlb-*	TET^I
ST7	*icaA-, icaD-, fnbpA-, embp-, aap-, hlb-*	FOX^R, ERY^R, TET^R	**ST31**	*icaA+, icaD-, fnbpA-, embp-, aap-, hlb-*	FOX^R, ERY^I, GEN^R, TET^R, CIP^R, $CO\text{-}TRI^R$
ST8	*icaA+, icaD+,fnbpA+,clfA+, sasG-, hlb+*	ERY^R, GEN^R, TET^R, CIP^R	**ST32**	*icaA+ icaD-, fnbpA+, embp-, aap-, hlb-*	FOX^R, ERY^R, GEN^R, TET^R, CIP^R, TOB^R, $CO\text{-}TRI^R$
ST9	*icaA+, icaD+,fnbpA+, clfA+, sasG-, hlb+*	TET^I	**ST33**	*icaA-, icaD-, fnbpA+, embp-, aap-, hlb+*	*Sensitive*
ST10	*icaA+, icaD+,fnbpA+, clfA+, sasG-, hlb+*	*Sensitive*	**ST34**	*icaA+, icaD+,fnbpA+, clfA+, sasG-, hlb+*	*Sensitive*
ST11	*icaA+, icaD+,fnbpA+, clfA+, sasG-, hlb+*	*Sensitive*	**ST35**	*icaA+, icaD+,fnbpA+, clfA+, sasG-, hlb+*	*Sensitive*
ST12	*icaA+, icaD+,fnbpA+, clfA+, sasG-, hlb+*	*Sensitive*	**ST36**	*icaA-, icaD-, fnbpA-, embp-, aap-, hlb-*	$CO\text{-}TRI^R$
ST13	*icaA+, icaD+,fnbpA+, clfA+, sasG-, hlb+*	*Sensitive*	**ST37**	*icaA-, icaD-, fnbpA-, embp-, aap-, hlb-*	ERY^R, GEN^R, TET^I, CIP^R, TOB^R, $CO\text{-}TRI^R$
ST14	*icaA+, icaD+,fnbpA+, clfA+, sasG-, hlb+*	ERY^I	**ST38**	*icaA-, icaD-, fnbpA-, embp-, aap-, hlb-*	FOX^R, ERY^R, GEN^R, CIP^R, RIF^R, $CO\text{-}TRI^R$
ST15	*icaA+, icaD+,fnbpA+, clfA+, sasG-, hlb+*	*Sensitive*	**ST39**	*icaA-, icaD-, fnbpA-, embp-, aap-, hlb-*	FOX^R, ERY^R, GEN^R, TET^R, CIP^R, RIF^R, TOB^R
ST16	*icaA+, icaD+,fnbpA+, clfA+, sasG-, hlb+*	*Sensitive*	**ST40**	*icaA+, icaD+,fnbpA+, clfA+, sasG-, hlb+*	*Sensitive*
ST17	*icaA+, icaD+,fnbpA+, clfA+, sasG-, hlb+*	*Sensitive*	**ST41**	*icaA-, icaD-, fnbpA-, embp-, aap-, hlb-*	*Sensitive*
ST18	*icaA+, icaD+,fnbpA+, clfA+, sasG-, hlb+*	*Sensitive*	**ST42**	*icaA+, icaD+,fnbpA+, clfA+, sasG-, hlb+*	RIF^I
ST19	*icaA+, icaD+,fnbpA+, clfA+, sasG-, hlb+*	*Sensitive*	**ST43**	*icaA-, icaD-,fnbpA+, clfA+, sasG-, hlb+*	*Sensitive*
ST20	*icaA+, icaD+,fnbpA+, clfA+, sasG-, hlb+*	*Sensitive*	**ST44**	*icaA+, icaD+,fnbpA+, clfA+, sasG-, hlb+*	*Sensitive*
ST21	*icaA+, icaD+,fnbpA+, clfA+, sasG-, hlb+*	*Sensitive*	**ST45**	*icaA+, icaD-, fnbpA-, embp-, aap-, hlb-*	*Sensitive*
ST22	*icaA+, icaD-, fnbpA-, embp-, aap-, hlb-*	GEN^R, TOB^R	**ST46**	*icaA-, icaD-, fnbpA-, embp-, aap-, hlb-*	TET^I, TOB^R
ST23	*icaA-, icaD-, fnbpA+, embp-, aap-, hlb+*	ERY^R, GEN^R, TOB^R, $CO\text{-}TRI^R$	**ST47**	*icaA+, icaD-, fnbpA+, embp-, aap-, hlb+*	*Sensitive*
ST24	*icaA-, icaD-, fnbpA+, embp-, aap-, hlb+*	ERY^R			

Table 15E. Antibiotic resistance profile of *Staphylococcus* spp. strains (ST48-ST142) isolated from medical devices. Only intermediate/resistance indicated. Tested antibiotics: **FOX:** Cefoxitin, **ERY:** Erythromycin, **GEN:** Gentamicin, **TET:** Tetracycline, **CIP:** Ciprofloxacin, **RIF:** Rifampicin, **TOB:** Tobramycin, **CO-TRI:** Cotrimoxazole.

Strain	Resistance profile	Strain	Resistance profile	Strain	Resistance profile
ST48	ERY^R	**ST92**	ERY^R, GEN^R, TOB^R, CO-TRI^R	**ST131**	FOX^R, ERY^R, GEN^R, CIP^R, TOB^R
ST49	ERY^R, TOB^R, CO-TRI^R	**ST94**	FOX^R, ERY^R, GEN^R, TET^I, CIP^R, TOB^R, CO-TRI^R	**ST132**	FOX^R, CIP^R, RIF^R, TOB^R
ST50	FOX^R, ERY^R, GEN^R, TET^I, CIP^R, RIF^I, TOB^R, CO-TRI^R	**ST96**	ERY^R	**ST133**	FOX^R, ERY^R
ST51	FOX^R, ERY^R, GEN^R, CIP^R, TOB^R, CO-TRI^R	**ST99**	FOX^R, ERY^R, GEN^R, CIP^R, RIF^R, TOB^R	**ST140**	FOX^R, ERY^R, GEN^R, CIP^R, TOB^R, CO-TRI^R
ST52	FOX^R, ERY^R, GEN^R, CIP^R, TOB^R, CO-TRI^R	**ST100**	FOX^R, ERY^R, GEN^R, CIP^R, TOB^R, CO-TRI^R	**ST141**	FOX^R, ERY^R, GEN^R, CIP^R, TOB^R, CO-TRI^R
ST53	ERY^R, GEN^R	**ST101**	FOX^R, ERY^R, GEN^R, CIP^R, TOB^R	**ST142**	FOX^R, ERY^R, GEN^R, CIP^R
ST56	ERY^R, CO-TRI^R	**ST104**	FOX^R, ERY^R, GEN^R, CIP^R, TOB^R, CO-TRI^R		
ST60	FOX^R, ERY^R, GEN^R, CIP^R, TOB^R, CO-TRI^R	**ST105**	FOX^R, ERY^R, GEN^R, CIP^R, TOB^R, CO-TRI^R		
ST61	FOX^R, ERY^R, GEN^R, CIP^R, TOB^R, CO-TRI^R	**ST106**	FOX^R, CIP^R, TOB^R		
ST64	ERY^R	**ST107**	FOX^R, ERY^R, GEN^R, CIP^R, TOB^R		
ST65	FOX^R, ERY^R, GEN^R, CIP^R, TOB^R, CO-TRI^R	**ST108**	FOX^R, ERY^R, GEN^R, CIP^R, TOB^R		
ST66	FOX^R, ERY^R, GEN^R, CIP^R, TOB^R, CO-TRI^R	**ST109**	FOX^R, CIP^R, RIF^R, TOB^R		
ST67	ERY^R, TOB^R	**ST110**	FOX^R, ERY^R, GEN^R, CIP^R, TOB^R		
ST71	TET^R	**ST112**	FOX^R, ERY^R, GEN^R, CIP^R, TOB^R, CO-TRI^R		
ST76	FOX^R, ERY^R, GEN^R, CIP^R, TOB^R, CO-TRI^R	**ST113**	FOX^R, ERY^R, GEN^R, CIP^R, TOB^R, CO-TRI^R		
ST77	TOB^R	**ST114**	FOX^R, ERY^R, GEN^R, CIP^R, TOB^R		
ST78	ERY^R, TOB^R	**ST115**	FOX^R, ERY^R, CIP^R, TOB^R		
ST79	TOB^R	**ST116**	ERY^R, GEN^R, CIP^R, TOB^R		
ST81	TOB^R	**ST118**	FOX^R		
ST83	TOB^R	**ST119**	FOX^R, ERY^R, CIP^R, RIF^R, TOB^R		
ST84	TOB^R	**ST120**	FOX^R, ERY^R, GEN^R, CIP^R, RIF^R, TOB^R		
ST85	TOB^R	**ST122**	ERY^R		
ST86	TOB^R	**ST126**	FOX^R, ERY^R, GEN^R, CIP^R, RIF^R, TOB^R		
ST87	TOB^R	**ST127**	FOX^R, ERY^R, GEN^R, CIP^R, RIF^R, TOB^R		
ST89	TET^I	**ST128**	ERY^R		

11. Hemolytic activity

Besides the biofilm formation ability and antibiotic resistance, 101/219 (46.12%) strains revealed a β-hemolytic activity while none strain exhibited α- hemolysis **(Figure 22A)**. It noticed that the β-hemolytic activity was found in the majority of the *Staphylococcus* spp. strains (66.43%) comparatively to other isolated bacteria; followed by *En. faecalis* (18.18%) **(Figure 22B)**. The β-hemolytic activity of ST9, ST16 and ST17 strains is illustrated in **figure 23**.

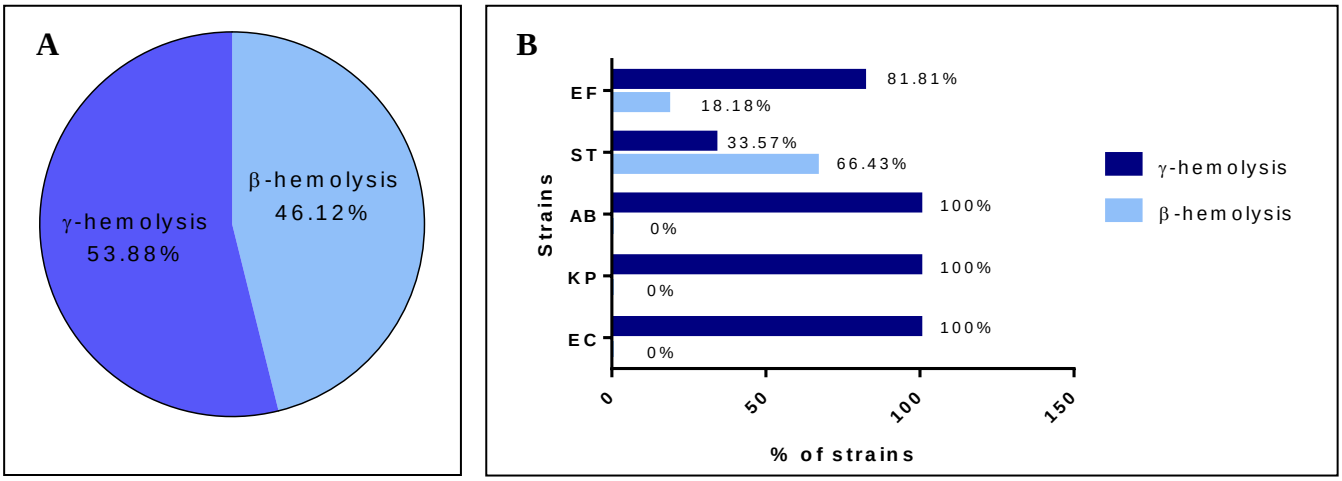


Figure 22. (A) Hemolytic activity type (%) of the isolated strains. **(B)** Distribution (%) of the strains according to hemolytic activity. **EC:** *E. coli*; **KP:** *K. pneumoniae*; **AB:** *Acinetobacter* spp.; **ST:** *Staphylococcus* spp.; **EF:** *En. faecalis.*

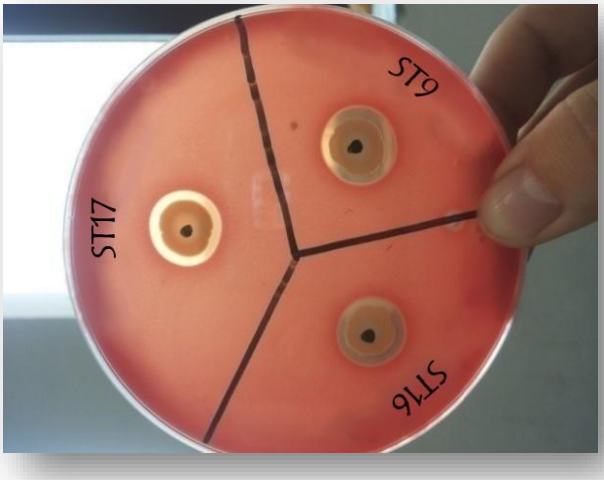


Figure 23. β-hemolytic activity of *Staphylococcus* ST9, ST16 and ST17 strains.

12. Bacterial cell surface Hydrophobicity

The results of the surface hydrophobicity of the isolated strains are depicted in **appendix IX**. The **figures 24 and 25** illustrate the different percentages of hydrophobicity of each bacterial

group obtained during the assay. The results showed that Gram- positive strains were more hydrophobic compared to Gram- negative ones. In fact, Gram- negative strains were either hydrophilic or moderately hydrophobic except *A. lwoffii* AL1 (59.80%) which was hydrophobic **(Appendix IX)**. For *Enterobacteriaceae*, 56.76% of the strains were moderately hydrophobic against 43.24% of hydrophilic strains **(figure 24)**. The *Enterobacteriaceae* strains showed a moderately hydrophobic character ranging from 20.10%±0.008 for EC4 strain to 48.91%±0.028 for EC26 strain **(Appendix IX)**. For *Acinetobacter*, 83.33% (5/6) of the strains were hydrophilic **(Figure 24, appendix IX**). Concerning *Staphylococcus*, 43.36% and 17.48% of the strains were hydrophobic and hydrophilic, respectively **(Figure 25)**. These strains showed a hydrophobic character ranging from 50.67%±0.011 for ST45 strain to 92.99%±0.025 for ST116 strain (**Appendix IX**). Among *En. faecalis* strains, 15.15% and 30.30% of them were hydrophobic (EF22=76.49±0.027) and hydrophilic, respectively, whereas more than half of the strains were moderately hydrophobic, ranging from 21.35%±0.023 for EF23 strain to 49.95%±0.025 for EF15 strain **(Figure 25, appendix IX**).

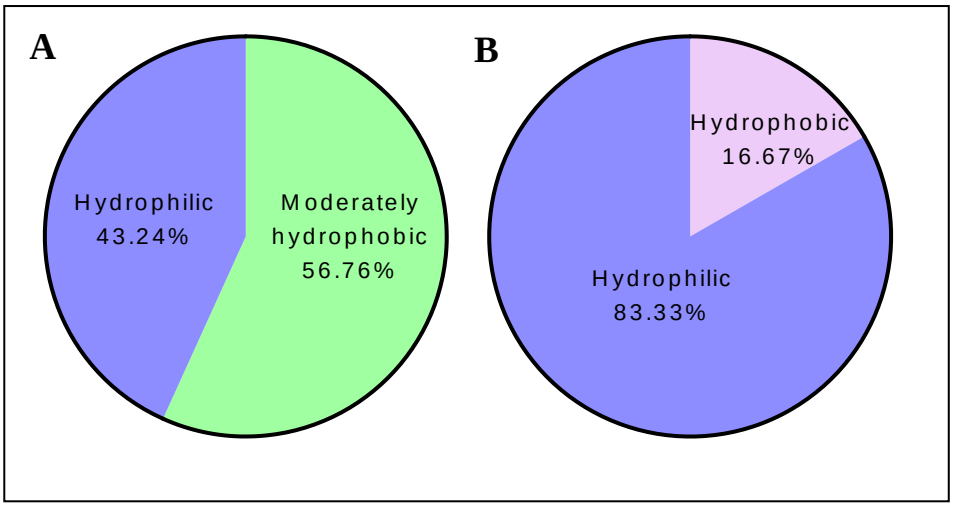


Figure 24. Percentages of *Enterobacteriaceae* **(A)** and *Acinetobacter* strains **(B)** according to their cell surface hydrophobicity

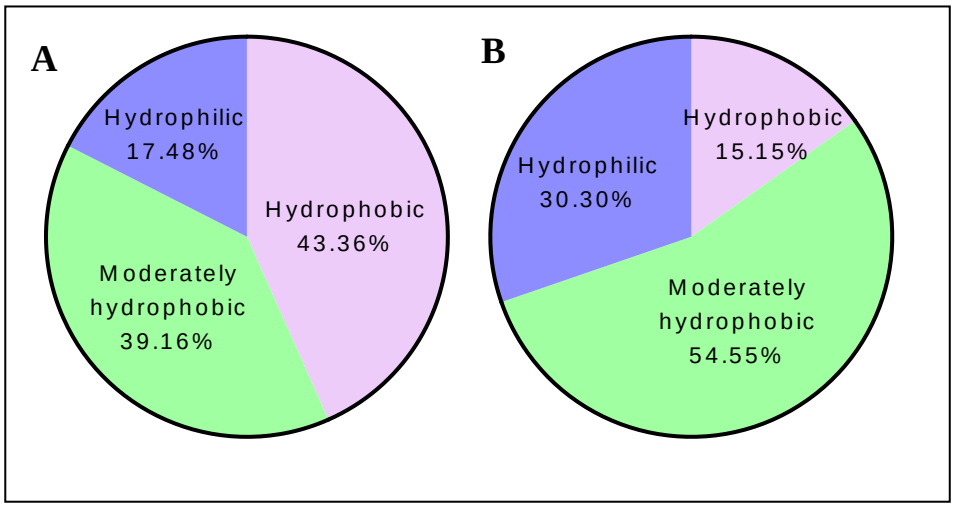


Figure 25. Percentages of *Staphylococcus* spp. **(A)** and *Enterococcus faecalis* **(B)** strains according to their cell surface hydrophobicity

13. Auto-aggregation and co-aggregation potential

The results of auto-aggregation of the isolated strains, which is an important step for adhesion and biofilm formation, showed variable rates of auto-aggregation **(Appendix IX)**. Out of 219 strains, 55 (25.11%) had auto-aggregation rates ≥20%. *E. coli* EC9 strain possessed the highest auto-aggregation ability (78.21%) compared to other strains. Interestingly, more than half strains (126/219) auto-aggregated with rates ranging from 10-20% **(Figure 26)**.

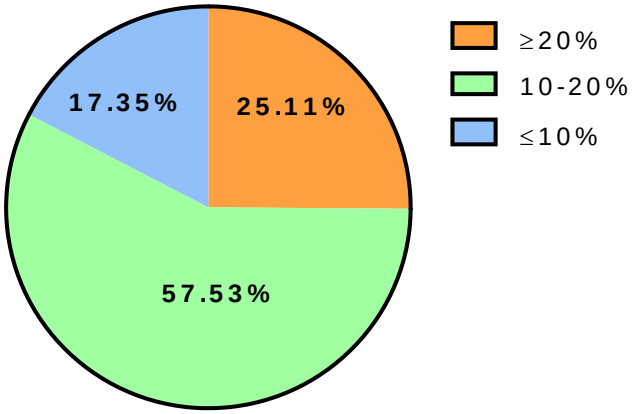


Figure 26. Auto-aggregation ability (%) of bacterial strains isolated from medical devices

The results of the co-aggregation assay between Gram- negative and Gram- positive isolated strains are given in the **appendix X**. The results showed variable percentages ranging from 1.26%±0.009 to 70.48%±0.024 for *E. coli* EC27+*En. faecalis* EF7 and *E. coli* EC28+ *Staphylococcus* ST29, respectively **(Appendix X)**. A co-aggregation with more than 50% was observed for the following combination strains: EC26+ST95 (52.50±0.026), EC19+ST140

(53.71±0.036), EC3+ST47 (54.50±0.038), EC23+ST97 (56.19±0.023), EC2+ST37 (56.62±0.037), AB1+ST40 (58.20±0.015), EC25+ST59 (69.43±0.033) and EC28+ST29 (70.48%±0.024) **(Appendix X)**.

14. Bactericidal efficacies of SAAP-148 and halicin on selected strains

Based on the above characterization data, four Gram-Negative Bacteria (GNB) (*E. coli* EC2, *A. baumannii* AB1, and *K. pneumoniae* KP1 and KP2), resistant to at least three different classes of antibiotics, were selected for further studies on the antibacterial properties of SAAP-148 and halicin. The results revealed that in a dose-dependent manner, SAAP-148 in PBS killed all four bacterial strains, with LC 99.9% values at concentrations ranging from sub-micromolar to low micromolar **(Table 16)**. In addition, in a dose-dependent manner, the peptide was effective against these bacteria in 50% (*v*/*v*) pooled human urine and 50% (*v*/*v*) human plasma, although 4–8× higher peptide concentrations were required. Similar bactericidal activities were obtained after 4 and 24 h of exposure to the peptide. Also, in a dose-dependent manner, halicin killed all four bacterial strains in PBS, with LC 99.9% values ranging between 6.4 µM and 51.2 µM depending on the strain **(Table 16)**. Halicin was more effective after 24 h than after 4 h of exposure. Of note, halicin was less effective against KP1, KP2, and EC2 in pooled urine than in PBS and virtually ineffective against AB1 in pooled plasma **(Table 16)**. These results revealed that the peptide SAAP-148 and halicin are potent candidates as antibacterial drugs against GNBs.

Table 16. Bactericidal efficacies of SAAP-148 peptide and halicin against antibiotic-resistant Gram-negative strains isolated from catheters.

Strains	Antibiotic Resistances											LC 99.9% of SAAP-148 (µM)				LC 99.9% of halicin (µM)			
												4 h		24 h		4 h		24 h	
	AMC	CTX	CAZ	FEP	FOX	IPM	TET	CIP	GEN	NA	CO-TRI	PBS	50% Plasma or Urine	PBS	50% Plasma or Urine	PBS	50% Plasma or Urine	PBS	50% Plasma or Urine
E. coli EC2												0.8	3.2	0.8	12.8	25.6 (12.8–25.6)	102.4 (51.2–102.4)	6.4	25.6 (12.8–25.6)
A. baumannii AB1												0.8	1.6	0.8	3.2	25.6	>102.4	25.6	>102.4
K. pneumoniae KP1												0.8 (0.8–1.6)	6.4	1.6	12.8	51.2 (25.6–51.2)	25.6	51.2	51.2
K. pneumoniae KP2												1.6 (0.8–1.6)	6.4 (3.2–6.4)	1.6	12.8	25.6 (12.8–25.6)	51.2 (25.6–51.2)	25.6	102.4 (51.2–102.4)

An *in vitro* killing assay was used to determine the bactericidal activities of SAAP-148 and halicin when administered in PBS; 50% urine (EC2, KP1, and KP2); and 50% human plasma (AB1). Approximately 2% (*v*/*v*) of the bacteria were mixed with increasing concentrations of SAAP-148 or halicin, and after 4 h and 24 h, the number of surviving bacteria was assessed microbiologically. The results are expressed as LC 99.9% values, i.e., the lowest concentration of the antimicrobial agent that results in a 99.9% reduction in the bacterial count compared to that of the control. The values are the means (and ranges) of six replicates from three independent experiments. If no range is mentioned, the LC 99.9% was identical in all the experiments. AMC: amoxicillin + clavulanic acid; CTX: cefotaxime; CAZ: ceftazidime; FEP: cefepime; FOX: cefoxitin; IMP: imipenem; TET: tetracycline; CIP: ciprofloxacin; GEN: gentamycin; NA: nalidixic acid; CO-TRI: cotrimoxazole; sensitive: green box; resistant/intermediate: red box; not tested: white box.

15. Reduction in bacterial counts in biofilms by SAAP-148 and halicin

Because biofilms are central to catheter-associated infections, we next determined the abilities of SAAP-148 and halicin to eradicate 24- h biofilms on a silicone elastomer, a surface mimicking a catheter. After 4 h of exposure, the BBC99.9 values for the peptide against EC2, AB1, KP1, and KP2 significantly amounted to 51.2 µM, 25.6 µM, 51.2 µM, and 51.2 µM, respectively; while after 24 h of exposure, they were higher (102.4 µM) for EC2 and AB1, BBC99.9= 51.2-102.4 µM and 51.2 µM for KP1 and KP2 **(Figure 27)**. Identically, halicin was effective against the four-strain biofilm and required highest concentration (BBC99.9= 102.4-204.8 µM). The agent was highly effective at a low concentration (~25.6 µM) against the EC2 biofilm after 24 h of exposure compared with the other strains, which required high concentrations (~102.4 µM) **(Figure 27)**. These data indicated that the compounds could be promising anti-biofilm agents.

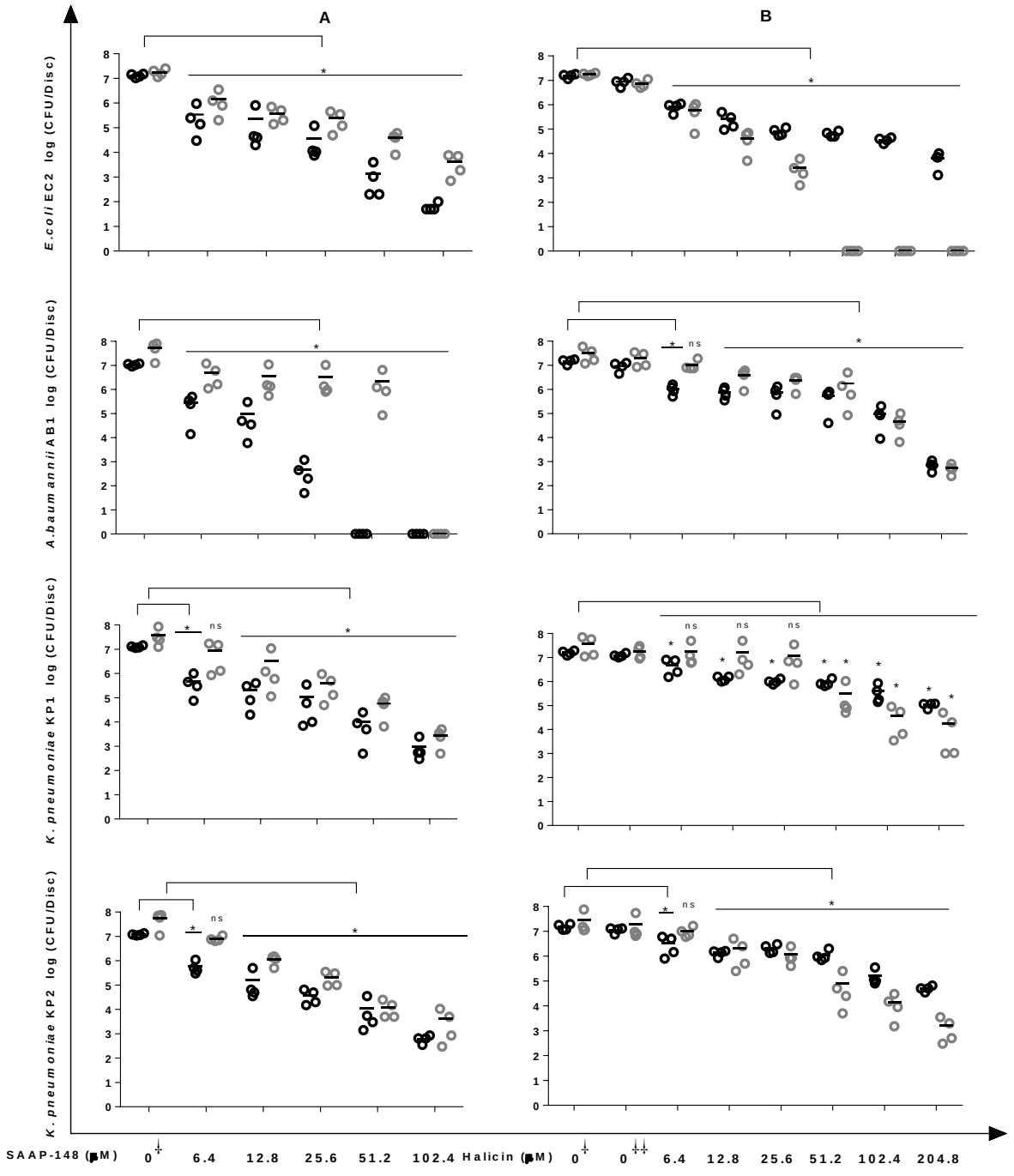


Figure 27. Reduction in bacterial counts within biofilms upon exposure to SAAP148 and halicin. The data obtained after 4 h and 24 h exposures are represented by open black and open grey symbols, respectively. Mann–Whitney U test: * $p < 0.05$ indicates significantly different from control ($0^{\downarrow}$, no agent). DMSO control ($0^{\downarrow\downarrow}$): control for halicin, i.e., the concentration of DMSO in the highest concentration of halicin that was tested. ns: not significantly different. Kruskal–Wallis test indicated that SAAP-148 as well as halicin at 4 h and 24 h were significantly effective against all the strains.

16. Effects of SAAP-148 and halicin on persisters derived from antibiotic-exposed mature biofilms

Because persisters play a pivotal role in chronic infections and antibiotic treatment failure, we assessed the effects of SAAP-148 and halicin on persisters derived from 7-day mature biofilms that had been exposed to high doses of antibiotics for an additional three days. As expected, high doses (50 × MBC) of ciprofloxacin significantly ($p < 0.01$) reduced (≥ 100×) the numbers of viable bacteria in the EC2, KP1, and KP2 biofilms, as did similarly high doses of gentamycin for the bacterial counts in the AB1 ($p < 0.01$) biofilms, without the complete elimination of all the bacteria **(Figure 28).** However, SAAP-148 was highly effective in killing persisters derived from antibiotic-exposed mature EC2, AB1, KP1, and KP2 biofilms **(Figure 28)**, with the complete eradication of the EC2 and AB1 persisters at 1.6 μM. For KP1 and KP2, slightly higher concentrations, i.e., 3.2 μM and 6.4 μM of the peptide were required, respectively **(Figure 28)**. Interestingly, the EC2 and AB1 persisters were eliminated by high concentrations of halicin, whereas the KP1 and KP2 persisters were not eradicated by this agent **(Figure 28)**.

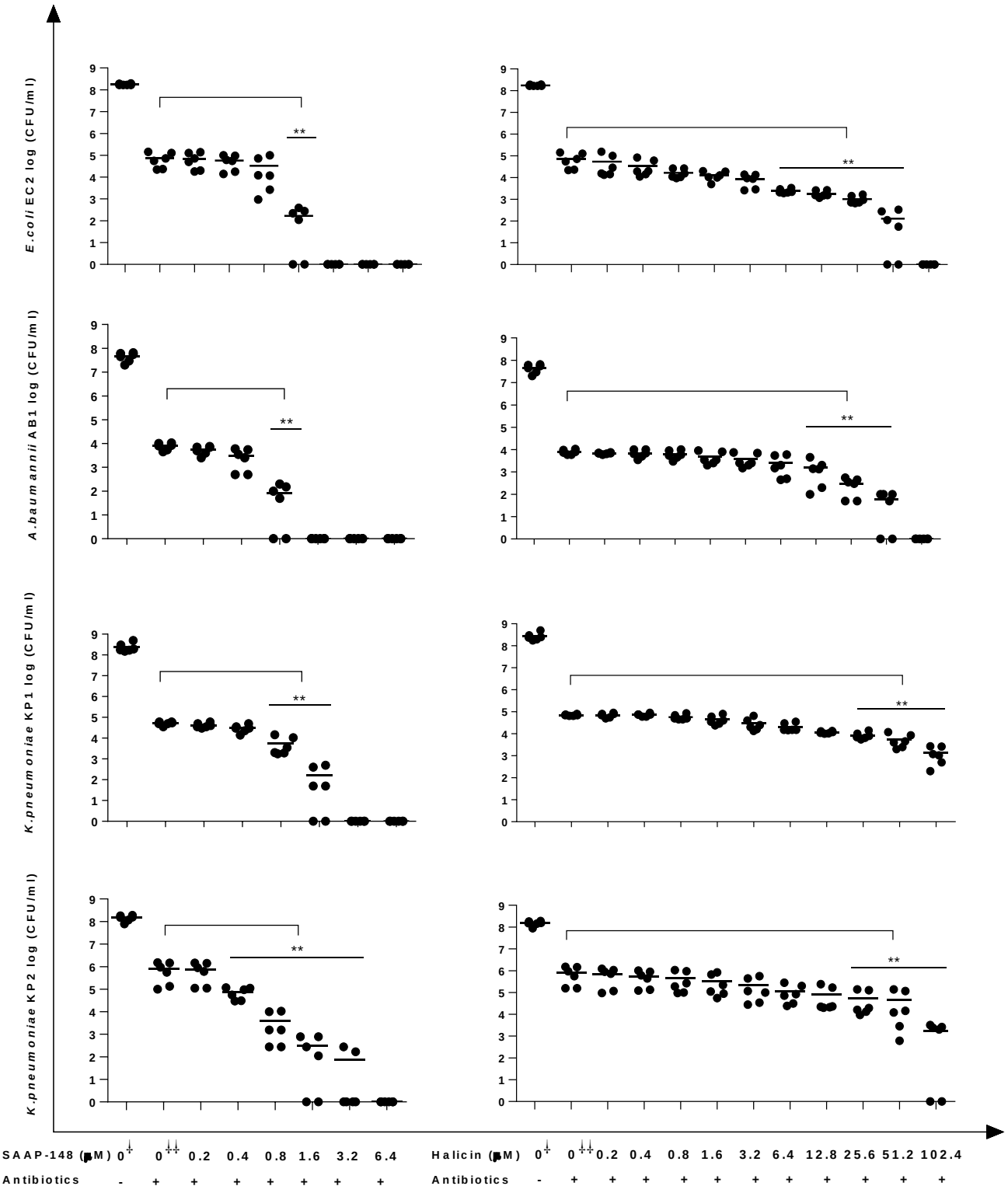


Figure 28. Effects of SAAP-148 and halicin on persisters derived from antibiotic-exposed mature biofilms. The Kruskal–Wallis test indicated that SAAP-148 and halicin were significantly effective against all the persisters at 4 h. $0^{\downarrow}$: number of bacteria before treatment with antibiotic. $0^{\downarrow\downarrow}$: number of bacteria after 3 days of exposure to the antibiotic. ** $p < 0.01$ (Mann–Whitney U test) indicated significant differences from controls exposed to the antibiotic ($0^{\downarrow\downarrow}$) but not SAAP-148/halicin.

17. Interactions between SAAP-148 and halicin

As the modes of action underlying the antibacterial activities of SAAP-148 and halicin differ **(de Breij *et al.*, 2018; Stokes *et al.*, 2020)**, the possibility of synergistic and/or additive interactions between these novel agents in eradicating bacteria in biofilms was investigated using checkerboard assays. The results revealed that several combinations of SAAP-148 and halicin were more effective than each agent alone in reducing bacterial counts in EC2, KP1, and KP2 biofilms but not in AB1 biofilms **(Table 17).** Based on the fractional biofilm eradication concentration index (ΣFBEC), some combinations of SAAP-148 and halicin acted synergistically on bacteria in EC2, KP1, and KP2 but not AB1 biofilms, whereas other combinations exerted additive effects **(Table 17).** Importantly, the concentrations of SAAP-148 needed to eliminate the bacteria in biofilms in the presence of halicin were considerably lower than that required for the peptide alone.

Table 17. Synergistic effects of SAAP-148 and halicin alone/in combination against GNB preformed biofilms and fractional biofilm eradication concentration indexes (ΣFBEC) for combinations of SAAP-148 and halicin.

Strain	MBEC SAAP-148 (μM) Alone	MBEC SAAP-148 (μM) in Combination	MBEC Halicin (μM) Alone	MBEC Halicin (μM) in Combination	ΣFBEC
E. coli EC2	102.4	3.2	25.6	6.4	0.28
		3.2		12.8	0.53
		6.4		12.8	0.56
		12.8		6.4	0.38
		12.8		12.8	0.63
		25.6		6.4	0.5
		25.6		12.8	0.75
A. baumannii AB1	102.4	No effect	102.4	No effect	No effect
K. pneumoniae KP1	51.2	12.8	102.4	12.8	0.38
		12.8		25.6	0.5
K. pneumoniae KP2	51.2	3.2	102.4	102.4	1.06
		12.8		12.8	0.38
		12.8		25.6	0.5
		12.8		51.2	0.75
		12.8		102.4	1.25
		25.6		51.2	1
		25.6		102.4	1.5

The experiment was assessed four times each in duplicate. Synergy is defined by the fractional biofilm eradication concentration index (ΣFBEC) ≤ 0.5; additive effect as 0.5 < ΣFBEC ≤ 1; indifference as 1 < ΣFBEC ≤ 2; antagonism as ΣFBEC > 2.

18. SAAP-148 and halicin neutralize GNB-induced IL-12p40 production by human blood leukocytes

We compared the abilities of SAAP-148 and halicin to reduce the capacities of EC2, AB1, KP1, and KP2 to stimulate the production of the pro-inflammatory cytokine IL-12p40 by human blood leukocytes. The results revealed that in a concentration-dependent manner, the pre-incubation of UV-inactivated bacteria with SAAP-148, but not halicin, reduced the ability of the strains to stimulate IL-12p40 production by blood leukocytes **(Figure 29)**.The concentrations of SAAP-148 that significantly ($p < 0.05$) reduced the abilities of UV-inactivated EC2, AB1, KP1, and KP2 to induce cytokine production amounted to ≥1 nM, ≥10 nM, ≥10 nM, and ≥10 nM, respectively **(Figure 29).** These preliminary data revealed that the synthetic peptide exhibited promising activity as an anti-inflammatory agent.

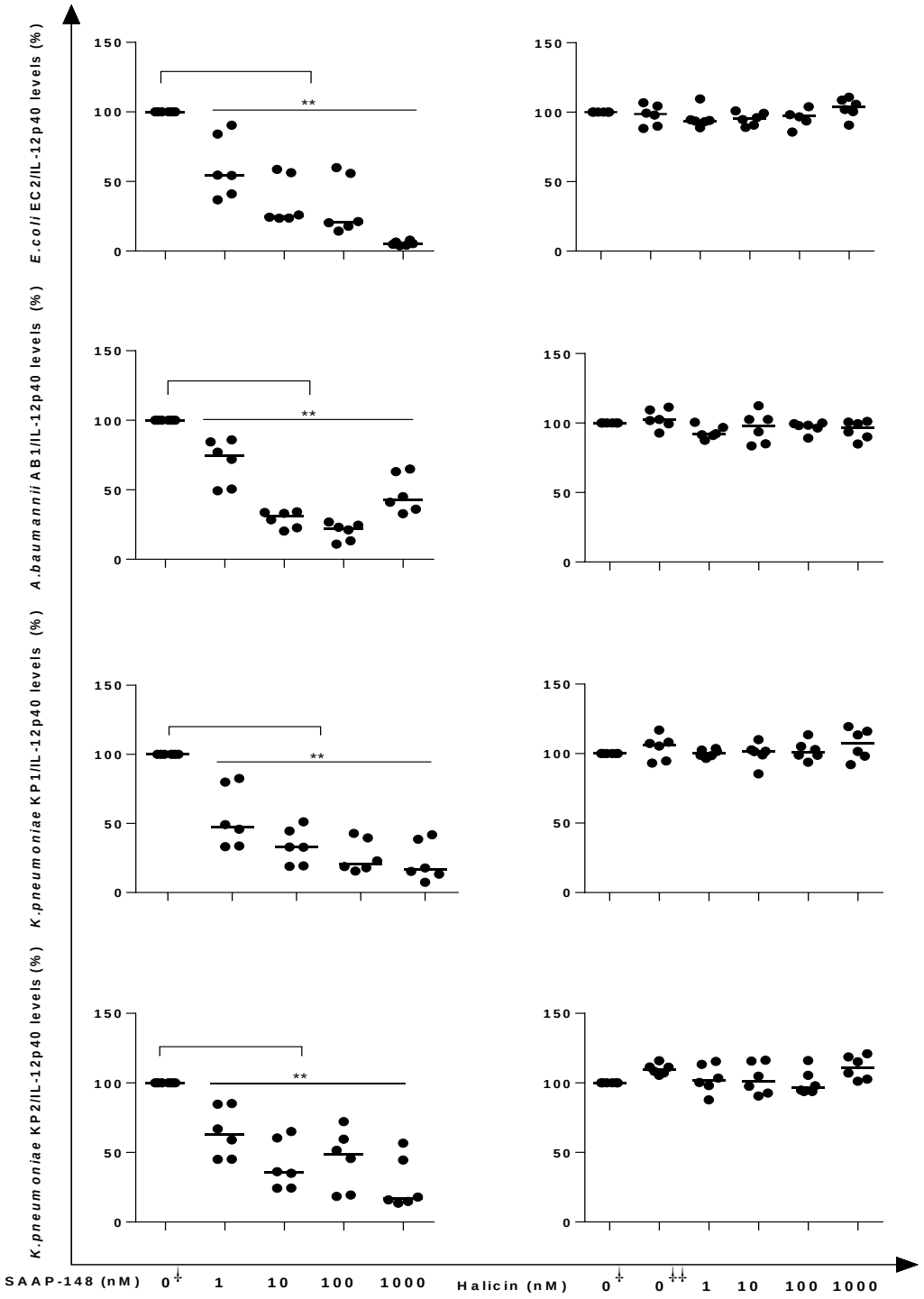


Figure 29. Pre-incubation for 2 h of UV-inactivated GNB strains with SAAP-148, but not halicin, reduced the ability of GNBs to induce IL-12-p40 production by whole human blood leukocytes. The results are expressed as the percentage of IL-12p40 compared with that of the control ($0^{\downarrow}$), i.e., UV-inactivated bacteria exposed to PBS instead of the peptide. $0^{\downarrow\downarrow}$: UV-inactivated bacteria exposed to the highest concentration of DMSO instead of the halicin concentration that was tested. Values are the means of three independent experiments, each performed in duplicate. The Kruskal–Wallis test indicated that SAAP-148 significantly reduced the abilities of all the bacteria (EC2 and AB1: $p < 0.0001$; KP1: $p = 0.0004$ and KP2: $p = 0.0005$) to induce IL-12p40 production. ** $p < 0.01$ compared to the control (no peptide) using Mann–Whitney U test.

19. Hemolytic activities of SAAP-148 and halicin

To obtain some insight into the *in vivo* toxicities of SAAP-148 and halicin, we assessed the hemolytic activities of these compounds using human erythrocytes suspended in 50% (***v/v***) human plasma and in PBS. The highest concentrations of SAAP-148 that lysed ≤5% of the erythrocytes in the PBS and 50% plasma amounted to <12.8 μM and 51.2 μM **(Figure 30A-B)**, respectively. The hemolytic activity of the halicin amounted to ≥204.8 μM in the PBS and 50% plasma **(Figure 30)**, respectively, indicating that halicin is less hemolytic than the peptide.

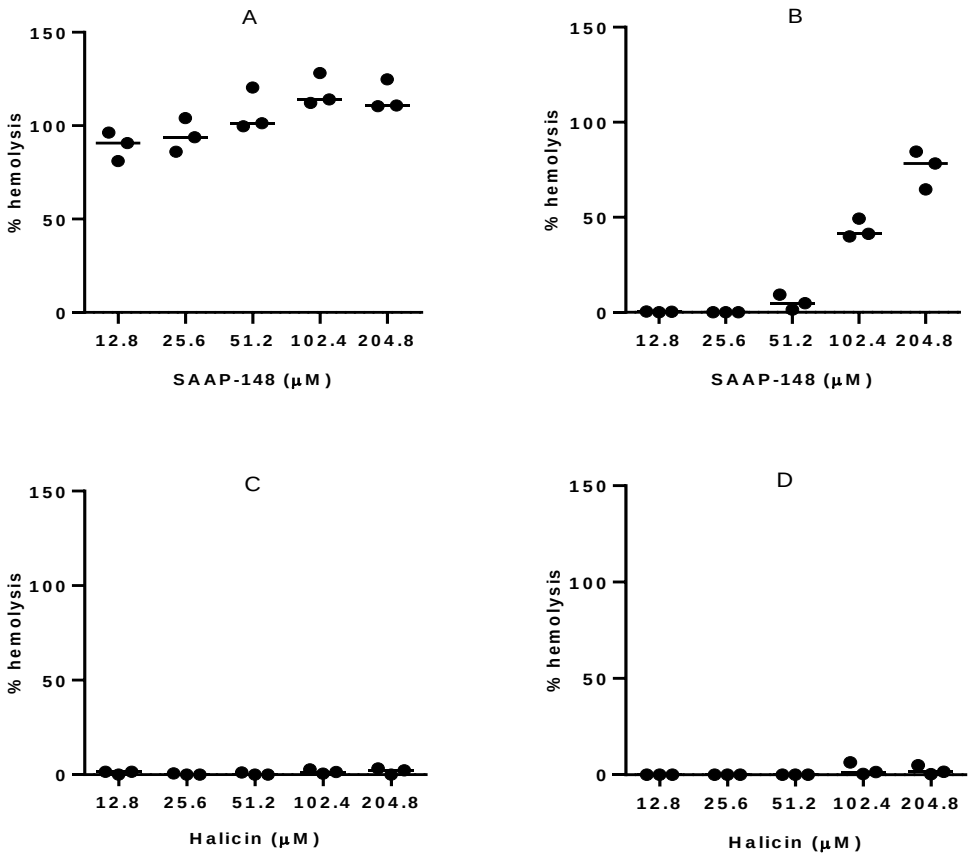


Figure 30. Hemolytic activities of SAAP-148 in PBS (**A**) and 50% plasma (**B**) and of halicin in PBS (**C**) and 50% plasma (**D**). Values are means of three independent experiments, each performed in triplicate.

General Discussion

The inappropriate use of medical devices is frequent and can increase the risk of bacterial infections **(Laan *et al*., 2020; Dougnon *et al*., 2023)**, leading to increased mortality and morbidity rates, prolonged hospitalization, overuse of antibiotics and exorbitant financial costs **(Gupta *et al*., 2023)**. In the current study, we were mainly interested in intravenous and urinary catheters (IVCs and URCs), the most devices used in healthcare settings **(Laan *et al*., 2020; Kochanowicz *et al*., 2022)**.

The present study is the first undertaken in Bejaia city (in the northeast of Algeria) and in Algeria concerning the use of novel antimicrobial agents (peptide SAAP-148 and halicin) and, thus, constitutes a precious source of information for the region and the country. A total of 40 medical devices (28 intravenous catheters [IVCs], 8 urinary catheters [URCs] and 4 intra-abdominal drains [IADs]) were collected from 39 patients (19 women and 20 men) hospitalized at least 48 h in two hospitals, and one private healthcare unit (Bejaia, Algeria).

Most medical devices were collected from General Surgery department (20 devices), followed by Internal Medicine department (13 devices), Resuscitation department (6 devices) and finally Infectious diseases department (1 device). In the current study, the average age of the patients was 47.4 years. The age group 17-34 years (35.9%) was the most frequent among patients from whom medical devices were collected. According to the medical case history of the patients, they presented different pathologies (e.g. peritonitis, gastric problems, diabetes, cancer and thrombosis…etc). It's important to underline that no local or general signs were observed in the patients from whom the medical devices were removed.

The IVCs were the most collected in General Surgery (13/20; 65%) and Internal Medicine departments (12/13; 92.3%), followed by Resuscitation department (3/6; 50%) and taken from men and women patients almost in equality (53.57% and 46.43%, respectively). The URCs were collected in General Surgery (3/20; 15%), Resuscitation (3/6; 50%), Internal Medicine (1/13; 7.7%) and Infectious diseases (1/1; 100%) departments and mostly from women (7/8; 87.5%). It is in compliance with the literature which states that each year, approximately 1.2 to 2 billion peripheral intravenous catheters (PVCs) are used in the world **(Dougnon *et al*., 2023; Zanella *et al*., 2024)**. Likewise, an estimated 100 million URCs are being used every year in the world **(Solomon, 2022; Al-Sayaghi *et al*., 2023)**. Moreover, the IVCs and URCs are often used for patients undergoing surgical procedures for medication

administration and prevention of incontinence in anaesthetized patients **(Meddings *et al*., 2019; Liu *et al*., 2020)**. Another medical device, called intra-abdominal drain (IAD) was also collected only in General surgery department (4/4; 100%) and taken from men. The IADs are often used after surgery (e.g. peritonitis by gastric ulcer perforation) or after intestinal anastomosis to prevent intra-abdominal infections **(Golash, 2009; Gilbert *et al*., 2018a)**.

The duration of placement of the recovered medical devices was mostly between 3-7 days (27 devices) and only 7 out of 40 were *in situ* for more than 7 days. It has been reported that a higher risk of infection development in patients is related with the increased duration of catheterization **(Gunardi *et al*., 2021)** and an average of three days catheterization was necessary for colonization and biofilm formation on central venous catheters (CVCs) **(Sharma *et al*., 2023)**. The duration of the implantation of the catheter for longer than 7 days increased the risk of infection **(Lafuente Cabrero *et al*., 2023)**. In addition, approximately 26% of patients having URCs for 2 to 10 days develop bacteriuria, and almost all patients having URCs for 1 month develop bacteriuria **(Gunardi *et al*., 2021)**. Overall, the poor health of patient (compromising of immune system), the advanced age of patient and the duration of catheterization are significant risk factors for the development of infections **(Shen *et al*., 2023; Lafuente Cabrero *et al*., 2023)**.

Concomitantly to URCs analysis, a urinalysis was done. Urinalysis, a routine, non-invasive and simple urine test to study the urine characteristics (color, pH, leukocytes, blood cells, epithelial cells, crystals, microorganisms, etc.) commonly performed in healthcare structures, gives information about the general health status of a patient, and is an important way to better understand potential pathologies related to the urinary system or others (renal and liver pathologies, diabetes, etc.) **(Haq and Patel, 2023; Milani and Jialal, 2023)**. The urine samples collected from patients with URCs were mostly cloudy, with a pH varying from 4.65 to 7.13. Normal urine has a pH of approximately 6.0 and can vary from 4.5 to 8.0 **(Manissorn *et al*., 2017)**. The leukocytes were positive (≥10 000/mL) in all the samples. However, the search for leukocytes in the urine of catheterized patients is not necessary because the URC itself can cause bladder inflammation **(Nicolle, 2014)**. The red blood cells were also observed in urine samples (ranged from 1000/mL to 12000/mL) and their presence can be explained by the fact that the friction between the catheter and urethral mucosa can damage the tissue causing bleeding **(Moore *et al*., 2023)**. Similarly, the presence of epithelium cells in urine may indicate the damage of bladder cells caused by the insertion of

the catheter **(Barford *et al*., 2008)**. Calcium oxalate crystals were also present in two urine samples where the pH was 5.82 and 7.13 respectively. It has been reported that the calcium oxalate can be formed at low urinary pH (<7.2) (**Shah *et al*., 2023)** and its high level increases the risk of kidney stones formation **(Carvalho, 2018)**. This demonstrates the adverse effects of catheterization on the bladder. Bladder irritation may be due to involuntary contractions of the bladder muscle and bad insertion of the urinary catheter by the healthcare staff **(Jang *et al*., 2020; Bhat *et al*., 2021)**, thereby causing burning sensation and pain in the suprapubic and penile areas leading to a discomfort for the patients and reduce the quality of their life **(Jang *et al*., 2020)**. To avoid these complications, a healthcare worker's education is needed and the procedure of bladder catheterization must be carefully studied before the intervention to warrant competency **(Haider and Annamaraju, 2023; Wills-Lee and Dunleavy, 2023)**.

A catheter-associated asymptomatic bacteriuria (CA-ASB) (colonization) is diagnosed by the presence of $\geq 10^5$ CFU/mL of microorganisms from a urine sample taken aseptically from patient with no symptoms related to a urinary tract infection (UTI) **(Nicolle, 2014)**. In the current study, the bacteriological examination of urine showed that 57.1% of patients had bacteriuria $\geq 10^5$ CFU/mL. It should be noted that, no local or general signs were observed in these patients with URC. In fact, catheterized patients with catheter- associated urinary tract infection (CA-UTI) usually do not present the classic symptoms related to UTIs (e.g. dysuria, frequent urination, and urgent urination) but may appear after removal of the catheter **(Hooton *et al*., 2010)**. In a study undertaken at Tlemcen hospital (Northwest, Algeria), nosocomial UTI was confirmed in all urine samples taken from 250 catheterized patients (143 women and 107 men; duration of insertion from 3 to 21 days) (**Kara Terki, 2014)**.

In our study, the diagnosis of colonization or infection related to these medical devices was carried out for 33 samples. The catheter-associated infection is based on a combination of a quantitative culture of the catheter $\geq 10^3$ CFU/mL (according to Brun-Buisson) and local and/or systemic symptoms, with their complete or partial resolution within 48 h after catheter removal. In contrary, the diagnosis of catheter colonization is based only on a quantitative culture $\geq 10^3$ CFU/mL, without any symptoms **(Christison *et al*., 2018; Timsit *et al*., 2020)**. On all analyzed medical devices, 36.4% (12/33) of them were colonized (absence of symptoms in all patients). Few Algerian reports about medical devices related infections (not colonization) are available. In the study of **Bellifa (2014)**, 66.7% (200/300) of patients with medical devices (URC, endotracheal and intubation tubes) were infected and 40% of

infections were related to URCs (80/200). A recent study conducted in Annaba University Hospital (Northeast, Algeria) showed that 27.54% of nosocomial infections were associated to the presence of medical devices (URC, IVC and gastric tube) **(Benzaid *et al*., 2022)**. The prevalence of healthcare- associated infections was 17.94% in Tlemcen University Hospital **(Chabni *et al*., 2017)**. Regarding other countries, a recent systematic review and meta-analysis, of 20 studies, demonstrated a high prevalence of CA-UTIs in Africa **(Asmare *et al*., 2024)**. **Pitiriga *et al*. (2023)** reported 10.28% of catheter colonization cases. Other studies reported also that 18% **(Choudhury *et al*., 2019)** and 11% **(Juhlin *et al*., 2021)** of PVCs were colonized with bacteria. It has been reported that PVC colonization is much more common **(Drugeon *et al*., 2024)**. In addition, the incidence of bloodstream infections associated with peripheral venous catheters (PVC-BSIs) is generally low, with a rate of 0.1% of short catheters inserted (0.5 episodes per 1000 days of intravascular catheter), unlike the incidence of CVC-BSIs which is 2.7 episodes per 1000 days of intravascular catheter **(Bertoglio *et al*., 2017; Sato *et al*., 2017)**. This incidence could be explained by the fact that PVCs are usually inserted in the hand and forearm, which are considered the two insertion sites with the lowest risk of dislodgement or kinking; and should be used for the catheters *in situ* for more than 2 days **(Buetti *et al*., 2022)**. On the other hand, in the current study, the most IVCs collected were PVCs which could explain our results.

Colonization of medical devices could result from the endogenous human microbiota or the hospital resident flora **(Neoh *et al*., 2017)**. In our study, *Staphylococcus* spp. were the predominant species in the IVCs (72%) (mostly coagulase- negative Staphylococci 107/121), followed by *Enterobacteriaceae* (15.5%), *Enterococcus* spp. (8.9%) and *Acinetobacter* spp. (3.6%). Other studies reported also the predominance of *Staphylococcus* isolates in IVCs with rates of 62% **(Pinto *et al*., 2022)**, 52.6% **(Dalai *et al*., 2018)**, 48.8% **(Osório *et al*., 2023)** and 16.9% **(Okafor *et al*., 2023)**, which is consistent with our finding. Coagulase- negative Staphylococci (CNS) isolates were the most found in the following studies: 44.4% **(Osório *et al*., 2023)** and 36.2% **(Pinto *et al*., 2022)**. However, in other studies, *S. aureus* was the most isolated strain from IVC: 39.47% **(Dalai *et al*., 2018)** and 16.9% **(Okafor *et al*., 2023)**. Given that *Staphylococcus* is considered as a commensally pathogen which is often found on the skin, this explains its high prevalence in IVCs **(Linz *et al*., 2023; Pimenta *et al*., 2023)**. Among Gram- negative bacteria (GNB), *E. coli* (~81%) and *A. baumannii* (16%) were the predominant species in the IVCs, and this could be explained by the local prevalence of these ubiquitous pathogens in the hospital environment **(Namiganda *et al*., 2019; Benamrouche *et***

***al*., 2020; Benzaid *et al*., 2022; Harzallah *et al*., 2022; Boulesnam *et al*., 2023)**. It is well documented that the hospital environment (e.g., bed rails, mattresses, medical equipment, colonized or infected patients, and the hands of healthcare workers) is an ecological niche for *A. baumannii* and *E. coli* **(Strasheim *et al*., 2015; Navab-Daneshmand *et al*., 2018; Daga *et al*., 2019)**. Although they are the second common bacteria, the focus on GNB, as causes of catheter -associated infections, should be not neglected as most epidemiological studies did **(Buetti *et al*., 2018)**. In fact, in recent years, the epidemiology and microbiology of catheter-associated infections have changed, and a shift in the predominance of their associated pathogens from Gram-positive to GNB, such as *E. coli*, *K. pneumoniae*, and *Acinetobacter* spp., is widely reported **(Tsuboi *et al*., 2019; Surapat *et al*., 2020; Rosenthal *et al*., 2020; Lendak *et al*., 2021; Mandolfo *et al*., 2022; Pitiriga *et al*., 2023; Pasilan *et al*., 2024).**

Similarly, *Staphylococcus* was the most recovered agent in 84.2 % IABs followed by *Enterobacteriaceae* (15.8 %; 2 *E. coli* and 1 *K. pneumoniae*). Moreover, IAD1 was poly-microbial with three different bacteria and IAD3 was mono-microbial. Several studies reported that Gram-positive bacteria (CNS, *S. aureus*, and *Enterococcus* spp.) were the most often isolated from surgical drain fluid **(Yoon *et al*., 2018; Chan *et al*., 2020; Pérez-Cameo *et al*., 2021)**,whereas other reports showed the predominance of GNB **(Yang *et al*., 2018; Kostakis *et al*., 2019; Basbulut and Kucuk, 2023)**. Drains are considered as foreign bodies, thus constituting a risk factor in the development of surgical site infections **(Puleo *et al*., 2013)**.

Regarding URCs, our data showed that *En. faecalis* strains were the most isolated agents (56.3%) followed by *Enterobacteriaceae* (25%), and *Staphylococcus* spp. (18.8%). In addition, among *Enterobacteriaceae*, *K. pneumoniae* was the most isolated species in this study (~56%), followed by *E. coli* (44%). Our findings are in agreement with the study of **Putta *et al.* (2018)** who found 40.62% of Enterococci, followed by *E. coli* (37.5%) and Staphylococci (12.5%), but not with other studies which reported that *E. coli* was the predominant agent in CA-UTIs **(Weiner *et al*., 2016; Köves *et al*., 2017; Dybowski *et al*., 2018; Peng *et al*., 2018; Gunardi *et al*., 2021)**. In these previous reports, *En. faecalis* was found in 7.4%, 14.9%, 14%, 15% and 13.1% of CA-UTIs, respectively. In the study of **Armbruster *et al.* (2017)** conducted in nursing homes, the predominance of *En. faecalis* (40%) in polymicrobial CA-UTIs was reported. Moreover, in the polymicrobial CA-UTIs, the interactions between the microorganisms would increase colonization and persistence

(Gaston *et al*., 2020). It has been reported that 65% of *E. coli* are involved in complicated UTIs **(Werneburg, 2022)** while *En. faecalis* contributes in 30% of CA-UTIs **(Codelia-Anjum *et al*., 2023)**. In Algeria, the predominance of *E. coli* in UTIs is widely reported **(Aouf *et al*., 2018; Nabti *et al*., 2019; Ait Mimoune *et al*., 2022)**, but no epidemiological data are available for catheter-colonizing uropathogens. However, two Algerian studies reported the presence of *K. pneumoniae* **(Bellifa *et al*., 2013)**; GNB and *Staphylococcus* isolates **(Kara Terki, 2014)** in URCs. It is not surprising to find these three uropathogens because *En. faecalis*, *E. coli* and *K. pneumoniae* are fecal contaminants or residents of patients' native or transient microflora that colonize the peri-urethral area **(Lengfelder *et al*., 2019; Flores-Mireles *et al*., 2019)**. In addition, the *Enterococcus* strains have the extraordinary ability to survive on several hospital surfaces for long time and in different harsh conditions (e.g. disinfection) which increase their persistence in healthcare settings **(Codelia-Anjum *et al*., 2023)**. It has been reported that early colonization of URCs usually involves *E. coli*, *Enterococcus* spp., *Staphylococcus* spp., *Klebsiella* spp. as well as other microorganisms **(Gaston *et al*., 2020).** Another observation in the current study is the presence of *Staphylococcus* spp. in URCs (18.8%). These bacteria can contaminate URCs surfaces via the skin flora of the patient and healthcare workers (hands) or through contaminated equipment **(Trautner and Darouiche, 2004; Papanikolopoulou *et al*., 2022)**.

To study the bacterial diffusion from biofilms formed on the medical devices collected from patients catheterized for more than one week, the roll-plate technique, which consists to roll colonized pieces of devices on the surface of blood agar plates, was used. A dense bacterial growth was observed for three devices (URC4, URC5 and IAD2). This method allows removing bacteria from the device **(Donlan, 2001a)** indicating their dispersion from medical devices. However, this technique is not able to remove bacteria from endoluminal colonization **(Slobbe *et al*., 2009)**. In fact, catheters left in place for less than 10 days form biofilms outside the catheter while catheters of 30 days and older generate biofilms more frequently inside the catheter **(Sharma *et al*., 2023)**.

To study the biofilm formation potential of the isolated strains, the tissue culture plate (TCP) method was used. As already reported **(Natsheh *et al*., 2023)**, the microtiter plate biofilm formation assay is the most widely used method to evaluate the adhesion of bacteria to a surface. As all the bacteria were derived from colonized catheters, we expected that 100% of the strains will form a biofilm on polystyrene surfaces. However, if this was true for

K. pneumoniae and *En. faecalis* isolates (100%), only 99.3% (142/143) of the *Staphylococcus*, 80.6% (25/31) of the *E. coli* and 83.3% (5/6) of the *Acinetobacter* isolates were biofilm producers in the tested conditions, which is in agreement with other studies where high ability of these strains in clinical to form biofilm is reported (**Ramírez Castillo *et al*., 2023; Sabença *et al*., 2023; Khoshnood *et al*., 2023; Dash *et al*., 2023; Asante *et al*., 2024; Nair *et al*., 2024).**

It's well documented that the characteristics of the surface play an important role in biofilm formation. Therefore, in further experiments, we used silicone elastomers, which more accurately mimic medical devices, such as venous and urinary catheters (**Pontes *et al*., 2016)**. On the other hand, Congo red agar (CRA) test which is a phenotypic and chromatic assay is another way to study biofilm formation **(Zafer *et al*., 2024)**. The ability of bacteria to form biofilm is correlated to the production of slime, an extracellular mucoid substance, polysaccharide in nature (glycosaminoglycans) **(Nasr *et al*., 2012)**. In our study, the CRA assay demonstrated that only 10% of the isolated strains were strong producers of slime. The strong production of slime was observed only in *Staphylococcus* strains with a rate of 15.38%. Among them, 68.18% and 31.81% were *S. aureus* and CNS, respectively. In the study of **Nasr *et al.* (2012)**, 50% and 45.5% of CNS and *S. aureus* isolates were strong producers of slime respectively. The production of slime was reported in other studies **(Hrv *et al.*,2016; Saba *et al.*, 2018; Karki *et al*., 2019; Achek *et al*., 2020; Jones *et al*., 2022; Asante *et al*., 2024)**. For example, the study of **Hrv *et al.* (2016)** demonstrated that 65.7% of CNS and 84% of *S. aureus* were strong slime producers and in the study of **Saba *et al.* (2018)**, 43% of *S. aureus* were CRA-positives. On the other hand, the obtained results showed that all *Enterobacteriaceae* strains (*E. coli* and *K. pneumoniae*), *Acinetobacter* spp. and *Enterococcus* spp. strains were non slime producers. Our results suggest that the CRA assay could not be the appropriate method to detect polysaccharides production in these strains, especially in *Acinetobacter* spp. isolates. Indeed, *Acinetobacter* spp. being non-fermentative bacteria, they are not able to produce secondary metabolites which will interact with Congo red to give the black color to the colonies **(Hrv *et al*., 2016; Zafer *et al*., 2024)**. Unfortunately, there are few data on the production of slime in GNB and *En. faecalis* isolates to which we can compare our results. In the study of **Karigoudar *et al.* (2010)**, 49% of *E. coli* strains were found to be slime producers. Another study showed that 78% of *E. coli* and 94.7% of *K. pneumoniae* were strongly positive by Congo red agar method **(Hrv *et al*., 2016)**. Recent studies showed that

slime production was positive for 63.08% of *En. faecalis* **(Kiruthiga *et al*., 2020)** and 26.9% of *Enterococcus* spp. **(Fahmy *et al*., 2021)**.

In addition to slime production, biofilm formation is mediated by multiple virulence factors, such as *fimbriae* (*fim*H) and curli (*csg*A), for *E. coli* **(Azam *et al*., 2020)**; CsuE, a subunit of the chaperone–usher pili (*csu*), the outer membrane protein (OmpA), and the biofilm-associated protein (Bap), for *A. baumannii* **(Colquhoun and Rather, 2020; Nie *et al*., 2020; Pompilio *et al*., 2021)** and *fimbriae* type 1 (*fim*H-1) and type 3 (*mrk*D), adhesin called "*E. coli* common pilus (*ecp*A), outer membrane lipoprotein (*ycf*M), for *K. pneumoniae* **(Ferreira *et al*., 2019; Vargas *et al*., 2019; Devanga Ragupathi *et al*., 2020)**. Similarly, several virulence factors are implicated in biofilm formation of Gram- positive bacteria, such as polysaccharide intercellular adhesion (PIA) (*ica*AD) **(Peng *et al*., 2022)**, fibronectin-binding protein A (*fnbp*A) and clumping factor A (*clf*A) **(Herman-Bausier *et al*., 2018; Speziale and Pietrocola, 2020)** and *S. aureus* surface protein G (*sas*G) **(Peng *et al*., 2022)**, for *S. aureus*; polysaccharide intercellular adhesion (PIA) (*ica*AD), extracellular matrix binding protein (*embp*) and accumulation–associated protein (*aap*), for CNS **(França *et al*., 2023)** and enterococcal surface protein (*esp*), aggregation substance (*asa*1), and gelatinase (*gel*E) **(Georges *et al*., 2022)**, for *En. faecalis*. The presence of these virulence factors in these hospital strains could increase the severity of infections **(Kiruthiga *et al*., 2020)**. In the present study, although these virulence genes were not fully detected in each isolated strain, some of them were harbored by all strains. Moreover, a comparison between the adhesion ability of the pathogenic strains and the virulence factors revealed that no correlation was observed between the presence/absence of these virulence genes and biofilm formation. However, a high negative correlation (r=-0.77) but not significant (p >0.05) was noted between the adhesion and the gene *csuE* in *Acinetobacter* spp. isolates. Our results are in concordance with some studies **(Bardbari *et al*., 2017; Saffari *et al*., 2017; Katongole *et al*., 2020; Silva *et al*., 2021; Chan *et al*., 2024)** and different from others **(Soares *et al*., 2014; Hamam *et al*., 2019; Monfered *et al*., 2019; Ballén *et al*., 2022b; Saadulla and Muhammed, 2023)**. Thus, we conclude that other virulence factors seem to be involved in biofilm formation.

Surprisingly, the *E. coli* strains were sensitive to almost all the antibiotics that were tested. These results are not in agreement with other Algerian studies reporting a prevalence of high resistance to β-lactams, gentamicin, and ciprofloxacin, except for imipenem, which

was active against *E. coli* strains **(Ayad *et al*., 2016; Bourafa *et al*., 2018; Nabti *et al*., 2019; Zenati *et al*., 2019)**. Our results showed that a high rate of biofilm-forming *E. coli* strains (83%) was more sensitive to antibiotics. Statistical analysis revealed no significant correlation (p >0.05) between their ability to form biofilm and their sensitivity to antibiotics, except for gentamycin where a significant moderate positive correlation (r=0.453; p=0.022) was recorded. This can be explained by the fact that the strains form biofilms to survive when exposed to antibiotics **(Shenkutie *et al*., 2020)**. Indeed, the polymeric matrix of the biofilm acts as a barrier and protects the bacteria from antibiotics, thereby preventing their penetration in the biofilm. In addition, the binding of antibiotics to matrix components may reduce the activities of the antibiotics **(Olivares *et al*., 2020; Topka-Bielecka *et al*., 2021; Zhao *et al*., 2023)**. **Alves *et al.* (2014)** reported that 73% of positive biofilm-forming *E. coli* isolates were sensitive to antibiotics and **Poursina *et al.* (2018)** also concluded that non-MDR *E. coli* strains were able to form strong biofilms.

We found that the *K. pneumoniae* strains harbored high rates of resistance to amoxicillin/clavulanic acid and ß-lactams (expect for cefoxitin), tetracycline, and gentamycin, while all the strains were sensitive to imipenem, nalidixic acid, ciprofloxacin (except for the KP5 strain), and cotrimoxazole. The majority of the Algerian studies used GNB clinical isolates, but a few Algerian studies enrolled catheter-colonizing strains **(Bellifa *et al*., 2013; Benzaid *et al*., 2022)**. High resistance to antibiotics has also been reported in other countries **(Osthoff *et al*., 2015; Ferreira *et al*., 2019; Ranjbar *et al*., 2019; Caneiras *et al*., 2019; Cusumano *et al*., 2019)**. In our study, 5/6 of *K. pneumoniae* produced an ESBL. Similarly, the presence of these ESBL producing strains were reported in few Algerian studies **(Berrazeg *et al*., 2013; Zennati *et al*., 2017; Zemmour *et al*., 2021)** and worldwild **(Müller-Schulte *et al*., 2020; Siriphap *et al*., 2022; Akenten *et al*., 2023; Sabença *et al*., 2023)**. It should be noted that these enzymes confer resistance to penicillin, aztreonam and first, second, and third-generation of cephalosporins, but are unable to hydrolyze cephamycins (cefoxitin) and carbapenems; and are usually found on transposons or insertion sequences of plasmids in association with other resistance genes causing multidrug resistance **(Castanheira *et al*., 2021; Husna *et al*., 2023)**. In addition, clinical *K. pneumoniae* ESBL-producing strains constitute one of the most worrying antibiotic resistance threats for human infections in Algeria **(Zemmour *et al*., 2021)**.

The *Acinetobacter* spp. strains exhibited high resistances to ß-lactams (expect for cefoxitin), imipenem, tetracycline, ciprofloxacin, and cotrimoxazole, but no strain was resistant to gentamycin, which is in agreement with antibiotic resistances in *A. baumannii* strains reported by other Algerian researchers **(Khorsi *et al*., 2015; Bakour *et al*., 2015; Bourafa *et al*., 2018; Benamrouche *et al*., 2020)**.

The majority of *S. aureus* strains was sensitive to all antibiotics tested (19/23; 82.16%). Only the strain ST8 was resistant to four antibiotics. Our results are in agreement with the reported Algerian studies **(Achek *et al*., 2018; Aouti *et al*., 2021)** and in disagreement with the study of **Namoune *et al.* (2023)** who reported that 64% of *S. aureus* isolates displayed multidrug resistance to several classes of antibiotics. High sensitivity of these strains was reported also in other countries **(Derakhshan *et al*., 2021, Su *et al*., 2023)**.

The CNS strains were found to be more resistant to the tested antibiotics which are not in agreement with the Algerian study of **Bouharkat *et al.* (2020)** where all the strains were susceptible to cefoxitin, gentamycin, erythromycin, except for tetracycline (16.7%). The study of **Zatout *et al.* (2020)** showed also high rates of resistance in the isolated strains with strong resistance to cefoxitin (80.3%). In other countries, high resistance rates were also reported **(Deyno *et al*., 2018; Nicolosi *et al*., 2020)**.

The *En. faecalis* strains exhibited high resistances to tetracycline, ciprofloxacin and erythromycin while 96.97% of strains were intermediate to cotrimoxazole. Only 15.15% of the strains were resistant to rifampicin. However, none strain was resistant to glycopeptides (vancomycin) and imipinem. Few Algerian studies reported also high antibiotic resistance profile of *En. faecalis* in clinical cases **(Rahmoun *et al*., 2020; Zerrouki *et al*., 2021)**. Concerning vancomycin, **Zerrouki *et al.* (2021)** and **Nair *et al*. (2024)** reported vancomycin resistance in 27.8% of isolates. A recent study demonstrated that 77.8% of their strains were sensitive to vancomycin. Other studies reported different antibiotic resistance patterns in *En. faecalis* isolates **(Said and Abdelmegeed, 2019; Boccella *et al*., 2021; Doss Susai backiam *et al*., 2023; Sannathimmappa *et al*., 2023)**.

Furthermore, the differences observed for the susceptibility profile of the pathogens to antibiotics in the different studies could be explained by the different locations of the clinical isolates (genetically different), different hospitals and different panels of antibiotics used. For

that, the therapeutic responses to antibiotics are different in regions of the same country and in different countries around the world **(Karigoudar *et al.*, 2019; Jafarzadeh Samani *et al.*, 2021).**

The hemolytic activity of the isolated strains, which is considered as another virulence factor, was tested in this study. In fact, hemolysis of red blood cells indicates the pathogenic power of bacteria and thus regularly studied **(Hrv *et al.*, 2016; Mogrovego *et al.*, 2020)**. Moreover, the hemolysis causes the releasing of hemoglobin and its breakdown product (free heme) **(Zhong and Yazdanbakhsh, 2018)**. The results showed that 46.12% of the strains (mostly *Staphylococcus* spp.) were β-hemolytic, whereas 53.88% of the strains were ɣ-hemolytic. The presence in combination of these different virulence traits (biofilm, hemolysins, and resistance to antibiotics) leads to persistent infections and makes treatment more difficult.

The attachment to biotic or abiotic surfaces is an important step for biofilm formation and cell surface hydrophobicity (CSH) is a relevant factor implicated in bacterial colonization **(Costa *et al.*, 2006; Nunez *et al.*, 2023).** Seeing that biomaterials such as intravenous catheters and urinary catheters, are made from hydrophobic materials (e.g. silicone), hydrophobic microorganisms are relatively easily adhering to them **(Krasowska and Sigler, 2014)**. In fact, cells with high CSH prefer hydrophobic surfaces while those with low CSH prefer hydrophilic surfaces **(Danchik and Casadevall, 2021)**. Our results showed that Gram-positive bacteria (*Staphylococcus* spp. 43.36% and *En. faecalis* 15.15%) had a higher affinity to xylen than Gram- negative bacteria (none strain), suggesting a hydrophobic character for them. A hydrophilic character was observed in 43.24% and 83.33% of *Enterobacteriaceae* and *Acinetobacter* spp., respectively. However, 95/219 strains displayed a moderately hydrophobic character (the most character observed). The presence of nonpolar molecules in bacterial membranes, including lipopolysaccharides, teichoic and lipoteichoic acids, and some surface proteins (S-layer) is responsible for their hydrophobic character **(Salas-Tovar *et al.*, 2021)**. Moreover, it is known that the outer membrane of GNB is rich in lipids (phospholipids, LPS) **(Horne *et al.*, 2020)**. However, our results showed that these bacteria are less hydrophobic. **Mirani *et al.* (2018)** reported that bacterial cells with high hydrophobicity produce strong biofilms, persist for a long time and are more difficult to disperse. It has been reported that the development of conditioning films, due to the adsorption of molecules such as proteins (e.g. albumin, fibrinogen, uromodulin), electrolytes

and polysaccharides from the surrounding environment such as blood and urine, modifies surface properties thus influencing the attachment of bacteria to biomaterials **(Lorite *et al*., 2011; Scotland *et al*., 2020; Bhagwat *et al*., 2021)**. Authors have shown that some proteins (e.g. albumin) can undergo a conformational reorientation (secondary, tertiary structure) in order to regulate their hydrophobic or hydrophilic characteristics during adsorption **(Duque-Sanchez *et al*., 2024)**. On the other hand, a recent study reported that loss of the hydrophilic bacterial capsule exposes the hydrophobic cell membrane. For this, non-encapsulated bacteria can easily adhere to hydrophobic surfaces **(Nunez *et al*., 2023)**. In fact, this group of researchers demonstrated that the loss of capsule in *K. pneumoniae* and *A. baumannii* improved their biofilm formation, probably by increasing cell surface hydrophobicity. In addition, the hydrophilic bacteria are able to modify the LPS or porines of their outer membrane **(Krasowska and Sigler, 2014)**. It should be noticed that, in addition to hydrophobicity, other factors influence bacterial adhesion to surfaces such as bacterial characteristics (charge, motility) and surface properties (surface charge, roughness, surface topography) and thus should be taken in consideration **(Zheng *et al*., 2021)**. For that, further studies to better understand the characteristics of the bacterial cells and the biomaterials surfaces are necessary.

Similarly, aggregation, or cell-cell interaction, is an important factor during biofilm development and involved in the beginning of biofilm formation steps. Furthermore, it has been reported that aggregation protects bacteria from external stresses (nutrient starvation, oxygen availability, temperature variability) and host immune system, and causes persistence of infections in the hospital environment **(Trunk *et al*., 2018; Gopinathan *et al*., 2021)**. We distinguish the auto-aggregation, which means the adherence of genetically identical cells and co-aggregation, which is the adhesion of genetically different cells **(Cai, 2020)**. In our study, only 55/219 (25.11%) strains showed auto-aggregation abilities with rates of ≥20%. We observed that the strain EC9 exhibited a highest auto-aggregation property (78.21%). Concerning co-aggregation, the results showed that only 8 combinations of Gram- negative and Gram- positive bacteria showed an ability of aggregation higher than 50%. Auto-aggregation is often mediated by surface proteins called autoagglutinins through homotypic interactions (e.g. polysaccharide intercellular adhesin, *fimbriae*, pili) **(Trunk *et al*., 2018).** The co-aggregation between two different bacteria is highly specific and involves lectin-like interactions (protein adhesin) and polysaccharide-containing receptors which can be found on

flagella and pili **(Stevens *et al*., 2015; Datta *et al*., 2017)**. In addition, co-aggregation is responsible for the formation of complex multispecies biofilm **(Datta *et al*., 2017)**.

As mentioned above, the hydrophobicity and auto-aggregation are implicated in biofilm formation **(Wang *et al*., 2021)**. However, no significant association ($p > 0.05$) between adhesion and hydrophobicity, adhesion and auto-aggregation, hydrophobicity and auto-aggregation was noticed. A significant low positive correlation (hydrophobicity *vs*. auto-aggregation, r=0.377, $p=0.031$) and moderate negative correlation (adhesion *vs*. auto-aggragation, r= -0.463, $p=0.006$) were observed in *En. faecalis* strains. The study of **Stępień-Pyśniak et al. (2019)** reported a significant positive correlation between hydrophobicity and biofilm formation in *En. faecalis* ($P = 0.00718$). Concerning the other strains, divergent results were found in other studies **(Di Martino *et al*., 2003; Kim *et al*., 2016; Iyer *et al*., 2023; Aleksic Sabo *et al*., 2024)**.

After the characterization of the isolated strains and demonstration of their biofilm formation potential, an evaluation of the antibacterial and anti-biofilm effects of two antimicrobial agents (SAAP-148 and halicin), alone or in combination, on GNB-colonizing catheters was performed. We used single strains of *E. coli* and *A. baumannii* and two *K. pneumoniae* strains. The results of the killing assay revealed that SAAP-148 and halicin are highly active against the tested strains. This observation is in agreement with earlier reports showing the effectiveness of SAAP-148 **(de Breij *et al*., 2018)** and halicin **(Stokes *et al*., 2020)** against a wide range of bacteria, including MDR- GNB (*E. coli*, *A. baumannii*, *P. aeruginosa*, and *K. pneumoniae*). Contrariwise, the activities of both agents were reduced in human plasma and urine. As already reported, the antimicrobial activities of peptides and antibiotics could decrease in the presence of these physiological fluids owing to the presence of components preventing the interaction of these agents with bacterial membranes **(de Breij *et al*., 2016; Nussbaumer-Pröll and Zeitlinger, 2020)**. In addition, SAAP-148 and halicin significantly reduced the bacterial counts within biofilms, and the required concentrations were considerably higher than those required for directly killing planktonic cells. This can be attributed to the biofilm matrix, which acts as a barrier that delays or prevents the interaction of antimicrobial agents with bacterial cells, thereby reducing the effectiveness of the agents against the biofilms **(Wu *et al*., 2021b)**. The results obtained with SAAP-148 are consistent with previous studies showing its efficacy against GNB- biofilms **(de Breij *et al*., 2018; van Gent *et al*., 2022)**. Notably, compared with the study of **van Gent *et al.* (2022)**, where a high

concentration (68.3 µM) of halicin was needed, this study revealed that halicin displayed the strongest effect on the *E. coli* biofilm at a low concentration (~25 µM). These findings suggest that the compounds have a promising anti-biofilm activity.

The presence of persister cells, which can survive in up to high doses of antibiotics, is mainly responsible for chronic infections and their recalcitrance **(Verderosa *et al*., 2019)**. In the present work, we exposed mature biofilms to high concentrations (50 × MBC) of ciprofloxacin (EC2, KP1, and KP2) and gentamycin (AC1) for 3 days. These antibiotics induced a deep disruption of the matrix, leading to their penetration of the biofilms **(Soares *et al*., 2019; Wang *et al*., 2019b)** and, thus, significantly reducing the number of bacteria within them. It has been suggested that the decrease in the activities of these antibiotics is correlated with persister cells **(Shenkutie *et al*., 2020)**. SAAP-148 and halicin were effective against these persisters derived from the antibiotic-exposed mature biofilms of the four tested bacterial strains in a dose-dependent manner. This is in agreement with earlier findings that SAAP-148 effectively reduced bacterial counts within antibiotic-exposed mature biofilms of MRSA **(Scheper *et al*., 2021; Verheul *et al*., 2021)**. Herein, we provided proof for the first time that SAAP-148 possesses a potent anti-persister effect against GNB- strains. Further, it should be kept in mind that planktonic, sessile, and persister bacteria all play important roles in CRIs, including catheter-related bloodstream infections and recurrent urinary tract infections **(Vestby *et al*., 2020; Bi *et al*., 2021)**.

Many antibiotics are known for their antibacterial activities but they are not able to suppress the pro-inflammatory effect caused by the LPS of GNB because of a lack capacity to neutralize these endotoxins, which induce a septic shock **(Wang *et al*., 2020c)**. For that, the development of molecules able to kill GNB without releasing their endotoxins (LPS), which are located in the outer membrane and induce severe pro-inflammatory responses, is a major challenge **(Jang *et al*., 2019; Gan *et al*., 2021)**. Peptides are able to bind to LPS and act as anti-inflammatory agents against endotoxic shock **(Heinbockel *et al*., 2018; Ridyard and Overhage, 2021)**. In our study, we found that SAAP-148, but not halicin, was able to reduce the ability of UV-killed GNB to induce IL-12-p40 cytokine production *in vitro*. Furthermore, these results are reported for the first time, indicating that this synthetic peptide could be an excellent candidate as an anti-inflammatory agent to treat sepsis caused by GNB infections. However, *in vivo* experiments to monitor organ injury in mouse models of LPS-induced endotoxemia and GNB-induced septic shock could give more evidences about the efficiency

of SAAP-148, as reported by **Jang *et al.* (2019)**. Several hypotheses on the mechanism of action of peptides that can induce an anti-inflammatory response have been reported. First, cationic peptides bind to LPS via electrostatic interactions, thereby neutralizing anionic amphiphilic lipid A. Second, the peptide binds to the macrophage's CD14 receptor and competitively inhibits the interaction of the LPS–LBP complex and, thus, blocks access to the TLR4 receptor, which mediates pro-inflammatory cytokines. Third, most LPS-binding peptides are able to dissociate LPS oligomers by depolymerization, thereby inhibiting LPS from binding to LBP **(Krishnan *et al*., 2021; Luo and Song, 2021)**.

In addition to antibacterial, anti-biofilm, and anti-inflammatory activities, low toxicity against human red blood cells (RBCs) is an important parameter for novel agents to be used as potential candidates in the treatment of biofilm-related infections **(Ebbensgaard *et al*., 2018)**. In fact, hemolytic activity constitutes the primary test for the *in vitro* study of toxicity to human erythrocytes **(Greco *et al*., 2020)**. We demonstrated that compared with halicin, which has no effect on RBCs, the peptide SAAP-148 is more toxic toward erythrocytes. The cytotoxicity assay, which is not included herein but is reported in a study by **van Gent *et al.* (2022)**, also showed that SAAP-148 was more cytotoxic than halicin for skin fibroblasts and RT-4 urethelial cells. It is known that hydrophobicity is crucial for peptides to disintegrate bacterial membranes. It has been reported that peptides possessing high hydrophobicity are correlated with high toxicity **(Guo *et al*., 2021; van Gent *et al*., 2022)**. The increasing hydrophobic content (tryptophan, lysine, and arginine) leads to a significantly high hemolytic activity **(Oddo and Hansen, 2017; Souza *et al*., 2020a)**. Moreover, SAAP-148 possesses a larger hydrophobic region in its amino-acid sequence **(Piller *et al*., 2022)**. The ability to form amphipathic structures has also been related to increased hemolytic activity **(Greco *et al*., 2020)**. Using SAAP-148 in combination with other agents could reduce the concentration of the peptide, thereby reducing the peptide's toxicity. Interestingly, recent studies have reported improved strategies for designing potent and safe peptides for therapeutics, such as PLGA (poly(lactic-co-glycolic acid)) nanoparticles **(Ali *et al*., 2023)** and C-terminal PEGylation **(van Gent *et al*., 2023)**, which seem to be promising approaches to overcome these drawbacks.

Furthermore, synergistic and additive interactions were noted between SAAP-148 and halicin against *E. coli* and *K. pneumoniae*, but not *A. baumannii*, in biofilms. These findings are in agreement with previous observations that the peptide and halicin act synergistically

against planktonic MRSA but not against MDR- *A. baumannii* **(van Gent *et al.*, 2022)**. Unfortunately, we cannot give an explanation for these species/strain-specific interactions between SAAP-148 and halicin. However, other researchers have reported similar findings for SAAP-148 and classical antibiotics **(Koppen *et al.*, 2019)** and for other antimicrobial peptides combined with other antimicrobial agents against GNB biofilms **(Gopal *et al.*, 2014; de la Fuente-Nuñez *et al.*, 2015; Jorge *et al.*, 2017; Swedan *et al.*, 2019; Kalsy *et al.*, 2020)**. Importantly, synergistic combinations of the peptide and halicin enable the use of lower doses of SAAP-148, possessing good antibacterial activities, while limiting harmful side effects *in vivo*. The use of antimicrobial peptides in combination with conventional/novel antibiotics or other antimicrobial agents could be an effective strategy to reduce the development of resistance **(Duong *et al.*, 2021)**. Future medical applications of (combinations of) SAAP-148 and halicin can be both invasive and non-invasive. Of note, combination treatment faces several hurdles, including the cytotoxicity and poor pharmakokinetic (PK)/pharmacodynamic (PD) properties of SAAP-148. However, conjugates of SAAP-148 and halicin could overcome several limitations encountered by the combinations. Regarding non-invasive usage, (combinations or conjugations of) SAAP-148 and halicin may be considered for antibiotic lock therapy **(van Praagh *et al.*, 2011; Signorino *et al.*, 2023)**, where (i) the catheter can be coated with the agents encapsulated in a polymeric formulation, such as hydrogel-loaded peptides and peptide-releasing hydrogels **(Riool *et al.*, 2017; Negut *et al.*, 2022; Copling *et al.*, 2023)**, or (ii) the agents may be immobilized on the surface of the catheter (**Riool *et al.*, 2017)**. The use of antimicrobial agents as a coating is the most popular approach owing to their ability to target microorganisms in different ways **(Andersen and Flores-Mireles, 2021)**. Several studies have reported the effectiveness of the use of antibiotics **(Gefter Shenderovich *et al.*, 2018; Menezes *et al.*, 2019; Srisang *et al.*, 2019)** and antimicrobial peptides **(Yu *et al.*, 2017; Monteiro *et al.*, 2019)** as coatings for eradicating/preventing the colonization of pathogens on catheter surfaces. Alternatively, the agents may be used invasively, e.g., immediately after the removal of a colonized catheter or as a treatment for patients with a CRI in whom the current treatment has failed.

It should be mentioned that the current *in vitro* study has some limitations. First, all the experiments were performed on a single strain of *E. coli* and *A. baumannii* and two *K. pneumoniae* strains from one hospital. Another limitation of this study is that the experiments were performed under static conditions (microtiter plate assays), whereas colonized catheters *in situ* are constantly subjected to a dynamic flow of blood or urine. This limitation may be

circumvented in future experiments using flow cells, e.g., a modified Robbin's device, in which a biofilm is formed in an environment of plasma or urine flowing under a constant shear (reviewed by **Subramanian *et al.*, 2020**).

General conclusion and outlooks

Medical devices such as venous catheters (VCs) and urinary catheters (UCs) are widely used in the healthcare settings. However, the implantation of these devices is often accompanied by complications. About 60 to 70% of nosocomial infections (NIs) are linked to biofilms. In fact, in addition to the global emergence of multidrug resistance, another complication is the ability of microorganisms to adhere to surfaces and form biofilms which protect them and help them to persist in the host. It makes these infections worse, causing their recalcitrance and therapeutic failure, thereby increasing the rate of morbidity, mortality, healthcare cost, and length of hospitalization.

The aim of the current study was firstly to characterize the pathogenic strains isolated from medical devices (intravenous catheters, urinary catheters and intra-abdominal drains) by the evaluation of their ability to form biofilm, hemolytic potential, hydrophobicity, aggregation, virulence genes content and antibiotic resistance profile. In the second part of this study we investigated the efficacy of two new antimicrobial agents (peptide SAAP-148 and halicin) alone or in combination; against Gram-negative bacteria (GNB) colonizing catheters in inhibition and/or eradication of biofilm and persistent cells.

The analysis of 33 medical devices (insertion≤7 days), collected from patients hospitalized at least 48 h in different departments (General Surgery, Internal Medicine, Resuscitation and Infectious diseases departments) at two hospitals and a private healthcare unit (Bejaia, Algeria), showed that 12 of them were colonized with a positive culture $\geq 10^3$ CFU/mL (without the presence of symptoms in patients) following the definition of catheter-associated infections. Despite that, their surveillance is very important in healthcare because the colonization is considered as the first step leading to infection.

A total of 219 strains were isolated from the medical devices. After phenotypic (Gram staining, colony appearance, biochemical, physiological, API galleries tests) and genotypic identification (16S rDNA sequencing), the most isolated strains were belonging to *Staphylococcus* spp. (24 *S. aureus*, 3 *S. haemolyticus*, 1 *S. cohnii* and 115 other coagulase negative Staphylococci), *Enterobacteriaceae* (31 *E. coli* and 6 *K. pneumoniae*), 33 *En. faecalis* and *Acinetobacter* spp. (5 *A. baumannii* and 1 *A. lwoffii*).

The study of the biofilm formation was evaluated *in vitro* using TCP and slime production on Congo red agar assays. The results showed that the adhesion of the strains on polystyrene microplates varied widely among the strains and 85.84% of them were strongly adherents. Otherwise, 10% of the strains were producers of slime and all of them were

Staphylococcus spp. Moreover, the TCP assay appears to be the best way to study biofilm formation. Indeed, specific PCR targeting virulence genes, involved in adhesion and biofilm formation, confirmed the adhesion ability of these strains.

The cell surface hydrophobicity and aggregation, two factors involved in biofilm formation, were also investigated. The majority of the strains was moderately hydrophobic and showed variable rates of auto- and co-aggregations potential, despite their biofilm formation potential.

On the other hand, *K. pneumoniae* and *A. baumannii* exhibited high levels of resistance to the antibiotics tested followed by coagulase negative *Staphylococcus* and *En. faecalis*. Furthermore, 5/6 of *K. penumoniae* strains were ESBL producers. The overuse of antibiotics in the hospital environment exerts a selection pressure on the emergence of MDR-strains. Surprisingly, the *E. coli* and *S. aureus* strains were sensitive to almost all the antibiotics. Likewise, 46.12% of the strains were β-hemolytic. All these virulence traits allow the adhesion and persistence of these pathogenic strains on medical devices and reduce the efficiency of antibiotics. Thus, the need of novel antimicrobial and anti-biofilm agents is urgent and synthetic peptides and antibiotics are the new therapeutic agents relied on.

So, in this study, the efficacy of two novel antimicrobial agents, i.e., the synthetic peptide SAAP-148 and the novel antibiotic halicin, against Gram-negative bacteria (GNB) colonizing catheters was evaluated. The bactericidal activity of SAAP-148 and halicin was reduced in urine and plasma as compared to buffered saline. In a dose-dependent manner, the both agents reduced bacterial counts in 24- h preformed biofilms on silicone elastomer discs and eliminated persisters originating from antibiotic-exposed mature 7-days biofilms, with halicin being less effective than SAAP-148. Importantly, SAAP-148 and halicin acted synergistically on *E. coli* and *K. pneumoniae* biofilms but not on *A. baumannii* biofilms. The peptide, but not halicin, decreased the production of IL-12p40 upon exposure to UV-killed bacteria.

This preliminary study showed that SAAP-148 and halicin are promising candidates to fight bacterial colonizing catheters and catheter- associated infections owing to their high broad-spectrum, antibacterial, anti-biofilm, anti-persisters, and anti-inflammatory activities *in vitro*; but importantly, to their ability to act synergistically on biofilm eradication, where their combined effect is superior to their individual effects.

It should be noted that, despite all the *in vitro* and *in vivo* studies that have been conducted in this area until now, no ideal strategy has been found until now due to the divergence of the results obtained and additional research studies are still needed to develop biocompatible and safer strategies and fully validate their efficiency in order to prevent and fight medical device-associated biofilm infections, especially for long-term catheters. Meanwhile, the risk of these infections could be reduced by respecting the prevention guidelines, including educating healthcare personnel, hygiene, limiting use of the catheters and antimicrobial lock therapy.

The future outlooks of this work are:

- The study of the effectiveness of the peptide SAAP-148 and halicin alone or in combination on mature biofilms (>7 -days biofilm);
- Prevention of biofilm formation on catheters (using silicone elastomer disc);
- The study of the effectiveness of the peptide SAAP-148 and halicin alone or in combination using human bladder and skin infection models; and in *in vivo* using mouse catheter infection models infected with pathogenic bacteria;
- Transcriptomic studies could be useful for understanding the expression of virulence genes involved in biofilm formation during catheter- associated infections before and after exposure to the antimicrobial agents;
- The design of more potent and safer therapeutic antimicrobial agents using several strategies, like chemical modification, nanotechnology-based delivery systems, and computer-aided design.

These outlooks deserve to be exploited at the level of our laboratory in collaboration with the Laboratory of Department of Infectious Diseases, Leiden University Medical Center (LUMC).

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Appendices

Appendix I

Table 1. Main medical device-associated Gram- negative bacteria and their virulence and adhesion factors implicated in biofilm formation.

Pathogen	Virulence factor	Characteristics and Function	Reference
E. coli	Type I *fimbriae*	Encoded by *fim* operon located on the chromosome of UPEC isolates; Binding specifically to D-mannose which is found on the glycoproteins of the epithelial cells; Represents 95% of the virulence factors of *E. coli*; A major adhesin in colonization of UCs and biofilm formation during CA-UTIs.	Reisner *et al*., 2014 ; Sarshar *et al*., 2020 ; Ghavidel*et al*., 2020
	P *fimbriae*	Pyelonephritis-associated pili (Pap); The second important adhesin expressed by UPEC and coded by the *pap* operon; Binding to di-galactoside moiety present in the urinary tract epithelium.	Jacobsen *et al*., 2008; Gupta *et al*., 2021; McLellan *et al*., 2021
	Curli Csg A	Key components of the extracellular biofilm matrix of *E. coli* in which CsgA is the major subunit of curli; Bacterial binding with fibronectin, laminin, plasminogen; Aggregation, adhesion to surfaces, and biofilm development.	Nhu *et al*., 2018; Luna-Pineda *et al*., 2019
	Hemolysin F	Encoded by the gene*hlyF;* Over-expression of *hlyF* promotes the biosynthesis of the outer membrane vesicles (OMVs) which release toxins involved in virulence.	Murase *et al*., 2016 ; vanderWesthuizen *et al*., 2019
	α-hemolysin	Encoded by the gene *hlyA;* A pore-forming cytotoxin, responsible for lysis of the cell membrane of hosts (leukocytes, erythrocytes, and endothelial cells).	Raheel *et al*., 2020
	LPS (Lipopolysacharide)	An endotoxin that induces septic shock caused by over-expression of pro-inflammatory cytokines.	Wang *et al*., 2020
K. pneumoniae	Types 1 *fimbriae*	Encoded by *fimAICDFGHK* operon; Adhesion mediation to mannose-containing structures present on host tissue and extracellular matrix; A major role in biofilm formation on UCs, invasion and colonization of host cells, and persistence in catheters-associated infections.	Stahlhut*et al*., 2012; Gomes *et al*., 2021
	Types 3 *fimbriae*	Encoded by *mrkABCD* operon; Adhesion to different structures in kidney, lung tissue, endothelial and bladder epithelial cells; A major role in biofilm formation on UCs, invasion and colonization of host cells, and persistence in catheter-associated infections.	
	Capsule polysaccharides (*magA*, *k2A* and *wcaG)*	Resistance to phagocytosis; Complement-mediated lysis inhibition and opsonization; Host defense escape.	Vargas *et al*., 2019; Riwu *et al*., 2022
	Lipopolysaccharides (*wabG*, *uge*, *ycfM)*	Inhibition of complement pathway; Inactivation of the seditious response; Block the effect of peptides via lipid A; Host defence escape.	
	Csu (Chaperone-usher pili)	Encoded by*csuA/BABCDE* operon; Involved in the initial ahesion onto abiotic surfaces but not biotic surfaces.	Colquhoun and Rather, 2020; Shenkutie *et al*.,

			2020
A. baumannii	OmpA (Outer membrane protein A)	A β-barrel porin, one of the most abundant porins in the outer membrane of *A. baumannii*. Role in the virulence of *A. baumannii*, including interaction with the host, cytotoxicity, apoptosis, and biofilm formation.	Lee *et al*., 2017; Colquhoun and Rather, 2020; Sarshar*et al*., 2021
	Bap (Biofilm-associated protein)	Formation of water channels; Maitainingthe structure and integrety of biofilm; Biofilm formation on abiotic and biotic surfaces.	Colquhoun and Rather, 2020; Pompilio*et al*., 2021
	Type V secretion systems	Transport exoproteins; Transport mobile genetic elements; Role in bacterial competition; Biofilm formation on abiotic and biotic surfaces.	Morris *et al*., 2019; Pompilio*et al*., 2021

Table 2. Main medical device-associated Gram- positive bacteria and their virulence and adhesion factors implicated in biofilm formation.

Pathogen	Virulence factor	Characteristics and Function	Reference
S. aureus	PIA (Intracellular adhesion polysaccharide) or PNAG (o poly-N-acetyl β-1-6 glucosamine).	Encoded by the *icaADBC* operon; Important in cell-to-cell adhesion, adhesion to surfaces, biofilm formation; Antimicrobial resistance; Bacterial protection from phagocytosis.	Arciola *et al*., 2015 ; Nguyen *et al*., 2020 ; Idrees *et al*., 2021
	FnBPA and FnBPB (Fibronectin-binding proteins)	Categorised as "microbial surface component recognising adhesive matrix molecules (MSCRAMM)"; Implication in binding host matrix components (fibronectin, fibrinogen, collagen, elastin, laminin); Initial cell attachment and/or biofilm formation; Implication in colonization; Immune evasion.	Magro *et al*., 2017; Moormeier *et al*., 2017 ; Idrees *et al*., 2021
	ClfA and ClfB (Clumping factors)		
	EbpS (Elastin binding protein)		
	Fib (Fibrinogen binding protein)		
	Atl (Autolysin)	Primary attachment through non-specific hydrophobic interactions with uncoated surfaces; Bindin to host extracellular matrix proteins and involvment in cell separation during cell division.	McCarthy *et al*., 2016; Hiltunen *et al*., 2019
	Bap (Biofilm-associated protein)	Contributionin initial adhesion to abiotic surfaces; Induction of strong intercellular adhesion.	Cucarella *et al*., 2001; Valle *et al*., 2020
S. epidermidis	PIA (Intracellular adhesion polysaccharide)	Adhesion and biofilm accumulation.	Oliveira *et al*., 2021
	AtlE (Autolysin E)	Attachment to plastic surfaces.	Otto, 2018
	Bap homolog protein Bhp	Adherence to a polystyrene surface, intercellular adhesion, and biofilm formation.	
	Ssp-1,Ssp-2 (Staphylococcal surface proteins 1 and 2)	Cell-to-cell adhesion; Biofilm formation.	
	Serine-aspartate repeat protein G (SdrG/Fbe) binding to fibrinogen	Adhesion to the host proteins (fibrinogen, collagen,fibronectin); Adhesion to abiotic surfaces.	Foster, 2020
	SdrF (Serine-aspartate repeat protein F) binding to collagen		

	Extracellular matrix-binding protein (Embp)		
En. faecalis	Esp (Enterococcal surface protein)	Primary adhesion in UTIs; Colonization of the urinary tract.	Stępień-Pyśniak *et al*., 2019; Codelia-Anjum *et al*., 2023
	Asa1 (Aggregation substance)	Adhesion to host cells and bacterial aggregation.	
	Collagen binding protein (Ace)	Adhesion to extracellular matrix and type 1 collagen.	
	Ebp A,B,C (Endocarditis and Biofilm-Associated Pili)	Important in initial attachment, biofilm formation, and endocarditis.	Madsen *et al*., 2017; Șchiopu*et al*., 2023
	CylA (Cytolysin A)	Killing other bacteria (especially Gram-negative bacteria) and eukaryotic cells (red blood cells); Biofilm formation.	Codelia-Anjum *et al*., 2023 ; Șchiopu*et al*., 2023; Gajewska *et al*., 2023
	Hyl (Hyaluronidase)	Degradation of hyaluronic acid to permeabilize host tissues; Induction of autoimmune diseases.	
	GelE (Gelatinase)	Degradation of the collagen adhesion protein (Ace) which contributes in colonization and biofilm formation; Degradation of gelatin, collagen, fibrin, fibrinogen, hemoglobin, and complement components	

Appendix II. API20E and APIStaph galleries

- **Principle**

API20E and APIStaph are standardized identification systems for *Enterobacteriaceae*, other non-fastidious Gram- negative rods and *Staphylococcus* species, respectively, which use 21 miniaturized biochemical tests and a database. The API strip consists of 20 microtubes containing dehydrated substrates. These microtubes are inoculated with a bacterial suspension that reconstitutes the media. During incubation, metabolism produces color changes that are either spontaneous or revealed by the addition of reagents. The reactions are read according to the reading table.

- **Identification by an API gallery can be summarized in three steps**

First, the preparation of a gallery consists of bringing together the bottom and the lid of an incubation box, then about 5 mL of distilled water are distributed into the honey-combed wells of the tray to create a humid atmosphere then the gallery is placed in the incubation box. Second, the preparation of a dense bacterial suspension by taking few colonies, made from pure and young culture (18-24 h old), and putting them in 5mL of an ampule of API Suspension Medium. Third, the filling of the gallery: both the tube and cupule of the tests CIT VP and GEL tests (API 20E) are filled with the bacterial suspension, but for the other tests, it is only the tubes (not the cupules). Anaerobiosis is created in the tests LDC, ADH, ODC, H2S, URE (API 20E)/ ADH, URE (API Staph) tests by filling the cupules with mineral oil. Finally, the incubation box is closed and incubated at 37°C for 18 to 24 h.

- **Results and interpretation**

After the incubation period, all the spontaneous reactionswill be read by referring to the reading table and recorded on the result sheet, and then the tests which require the addition of reagents (TDA, IND and VP tests for API 20E/ VP and NIT tests for API Staph) will be revealed.

Table 1. Identification table for API 20E.

Test	Active ingredients	Reactions/Enzymes	Reagent added	Result	
				–	+
ONPG	2-nitrophenyl ßD-galactopyranoside	ß-galactosidase	Direct reading	Colorless	Yellow
ADH	Arginine	Arginine dihydrolase	Direct reading	Yellow	Red/orange
LDC	L-lysine	Lysine décarboxylase	Direct reading	Yellow	Red/orange
ODC	L-ornithine	Ornithine decarboxylase	Direct reading	Yellow	Red/orange
CIT	Sodium citrate	Citrate utilization	Direct reading	Pale green/yellow	Blue-green/blue
H_2S	Sodium thiosulfate	H_2S production	Direct reading	Colorless	Black deposit
URE	Urea	Urease	Direct reading	Yellow	Red/orange
TDA	L-tryptophan	Tryptophane desaminase	Add 1 drop ofIron Perchloride (TDA reagent)/immediate	Yellow	Reddishbrown
IND	L-tryptophan	Indole production	Add 1 drop ofKovacs reagent/immediate	Colorless/Yellow	Pink
VP	Sodium pyruvate	Acetoin production	add 1 drop of KOH (VP1) and α-napthol (VP2)/10min	Colorless	Pink/red
GEL	Gelatin	Gelatinase	Direct reading	No diffusion	Diffusion of black pigment
GLU	D-glucose	F/O of glucose	Direct reading	Blue-green/blue	Yellow
MAN	D-mannitol	F/O of D-mannitol	Direct reading	Blue-green/blue	Yellow
INO	Inositol	F/O of inositol	Direct reading	Blue-green/blue	Yellow
SOR	D-sorbitol	F/O of D-sorbitol	Direct reading	Blue-green/blue	Yellow
RHA	L-rhamnose	F/O of L-rhamnose	Direct reading	Blue-green/blue	Yellow
SAC	D-sucrose	F/O of D-sucrose	Direct reading	Blue-green/blue	Yellow
MEL	D-melibiose	F/O of D-melibiose	Direct reading	Blue-green/blue	Yellow
AMY	Amygdaline	F/O of amygdaline	Direct reading	Blue-green/blue	Yellow
ARA	L-arabinose	F/O of amygdaline	Direct reading	Blue-green/blue	Yellow
NO_2	Nitrates	Reduction of nitrates to Nitrites (NO_2)	Add 1 dropp of NIT1 and NIT2 to the GLU tube/2 to 5 min	Yellow	Red

F/O: Fermentation or oxidation.

Table 2. Identification table for API Staph.

Test	Active ingredients	Reactions/Enzymes	Reagent added	Result	
				–	+
0	No substrate	Negative control	/	Red	/
GLU	D-glucose	Positive control (D-glucose)	Direct reading	Red	Yellow
FRU	D-fructose	Acidification (D-fructose)	Direct reading	Red	Yellow
MNE	D-mannose	Acidification (D-mannose)	Direct reading	Red	Yellow
MAL	D-maltose	Acidification (D-maltose)	Direct reading	Red	Yellow
LAC	D-lactose	Acidification (D-lactose)	Direct reading	Red	Yellow
TRE	D-trehalose	Acidification (D-trehalose)	Direct reading	Red	Yellow
MAN	D-mannitol	Acidification (D-mannitol)	Direct reading	Red	Yellow
XLT	Xylitol	Acidification (Xylitol)	Direct reading	Red	Yellow
MEL	D-melibiose	Acidification (D-melibiose)	Direct reading	Red	Yellow
NIT	Potassium nitrate	Reduction of nitrates to nitrites	add 1 drop of NIT1 and NIT2/10min	Colorless/light pink	Red
PAL	ß-naphtyl phosphate	Alkaline phosphatase	add 1 drop of ZYM A and ZYM B/10min	Colorless/Very light violet	Violet
VP	Sodium pyruvate	Acetoin production	add 1 drop of KOH (VP1) and α-napthol (VP2)/10min	Colorless/light pink	Violet/Pink
RAF	D-raffinose	Acidification (D-raffinose)	Direct reading	Red	Yellow
XYL	D-xylose	Acidification (D-xylose)	Direct reading	Red	Yellow
SAC	D-saccharose	Acidification (Sacchrose)	Direct reading	Red	Yellow
MDG	Methyl-αD-Glucopyranoside	Acidification (Methyl-αD-Glucopyranoside)	Direct reading	Red	Yellow
NAG	N-acetyl-glucosamine	Acidification (N-acetyl-glucosamine)	Direct reading	Red	Yellow
ADH	L-arginine	Arginine DiHydrolase	Direct reading	Orange/Red	Yellow
URE	Urea	Urease	Direct reading	Red/violet	Yellow

Appendix III

Table 1. Concentrations of SAAP-148 and halicin tested in the checkerboard.

Strain	**24-h treatment**			
	Final concentration SAAP-148 (µM)	**Final concentration Halicin (µM)**	**Control SAAP-148(µM)**	**Control Halicin (µM)**
***E. coli* EC2**	3.2-25.6	3.2-12.8	3.2-102.4	3.2-51.2
***A. baumannii* AB1**	3.2-25.6	12.8-51.2	3.2-102.4	12.8-204.8
***K. pneumoniae* KP1**	3.2-25.6	12.8-102.4	3.2-102.4	12.8-204.8
***K. pneumoniae* KP2**	3.2-25.6	12.8-102.4	3.2-102.4	12.8-204.8

Appendix IV

Table 1. General characteristic of the studied patients (IVC1-IVC11).

Patient	Age	Gender	Hospital	Admission department	Reason for admission	Medical device used	Duration *in situ*	Pathology that motivated the use of the device	Ongoing treatment	Symptoms before device removal
IVC1	45	Man	Hospital 1	General Surgery	Multinodular goiter	Peripheral venous catheter	5 days	Surgery	Antibiotics	No
IVC2	85	Woman	Private healthcare unit	General Surgery	Chronic cystitis	Peripheral venous catheter	7 days	Medicine	Antibiotics	No
IVC3	40	Man	Hospital 1	General Surgery	Stomach cancer	Peripheral venous catheter	3 days	Rehydratation	Serum	No
IVC4	73	Man	Private healthcare unit	General Surgery	Herniated disc	Peripheral venous catheter	3 days	Surgery	Anti-inflammatory	No
IVC5	38	Man	Hospital 1	General Surgery	Epigastric penetrating stab wound	Peripheral venous catheter	4 days	Surgery	Antibiotics	No
IVC6	66	Man	Hospital 1	General Surgery	Gallbladder lithiasis	Peripheral venous catheter	4 days	Surgery	Antibiotics	No
IVC7	31	Woman	Hospital 1	General Surgery	Peritonitis (Gastric ulcer perforation)	Peripheral venous catheter	3 days	Surgery	Antibiotics	No
IVC8	27	Man	Hospital 1	Resuscitation	Car accident	Peripheral venous catheter	2 days	Medicine	Antibiotics	No
IVC10	74	Man	Hospital 1	General Surgery	Pyloric stenosis	Peripheral venous catheter	5 days	Rehydratation	Serum	No
IVC11	51	Man	Private healthcare unit	General Surgery	Peritonitis (Gastric ulcer perforation)	Peripheral venous catheter	2 days	Surgery	Antibiotics	No

Table 2. General characteristic of the studied patients (IVC12-IVC20).

Patient	Age	Gender	Hospital	Admission department	Reason for admission	Medical device used	Duration *in situ*	Pathology that motivated the use of the device	Ongoing treatment	Symptoms before device removal
IVC12	69	Man	Hospital 1	General Surgery	Colon cancer	Jugular venous catheter	4 days	Surgery	Antibiotics	No
IVC13	52	Man	Hospital 1	General Surgery	Gallbladder surgery	Peripheral venous catheter	4 days	Surgery	Antibiotics	No
IVC14	22	Man	Private healthcare unit	General Surgery	Acute appendicitis	Peripheral venous catheter	4 days	Surgery	Antibiotics	No
IVC15	51	Woman	Hospital 1	Internal Medicine	Gastric problem	Peripheral venous catheter	3 days	Medicine	Antibiotics	No
IVC16	36	Woman	Hospital 1	Internal Medicine	Gastrointestinal bleeding	Peripheral venous catheter	5 days	Medicine	Antibiotics	No
IVC17	74	Man	Hospital 1	Internal Medicine	Prostate	Peripheral venous catheter	3 days	Medicine	Antibiotics	No
IVC18	55	Woman	Hospital 1	Internal Medicine	Leukemia (Chemotherapy)	Peripheral venous catheter	6 days	Medicine	Antibiotics	No
IVC19	67	Woman	Hospital 1	Resuscitation	Severe hyperglycemia	Peripheral venous catheter	2 days	Coma	Antibiotics	No
IVC20	22	Woman	Hospital 1	Internal Medicine	Crohn’s disease	Peripheral venous catheter	3 days	Medicine	Antibiotics	No

Table 3. General characteristic of the studied patients (IVC21-URC3).

Patient	Age	Gender	Hospital	Admission department	Reason for admission	Medical device used	Duration *in situ*	Pathology that motivated the use of the device	Ongoing treatment	Symptoms before device removal
IVC21	32	Man	Private healthcare unit	General Surgery	Stab wound	Peripheral venous catheter	2 days	Surgery	Antibiotics	No
IVC22	21	Woman	Private healthcare unit	Internal Medicine	Diabetic cytosis	Peripheral venous catheter	3 days	Medicine	Antibiotics	No
IVC23	17	Woman	Hospital 1	Internal Medicine	Breast surgery	Peripheral venous catheter	3 days	Medicine	Antibiotics	No
IVC24	51	Woman	Hospital 1	Internal Medicine	Neurological problem	Peripheral venous catheter	3 days	Medicine	Anti-inflammatory	No
IVC25	18	Man	Hospital 1	Internal Medicine	Gastric problem	Peripheral venous catheter	2 days	Medicine	Serum	No
IVC26	17	Woman	Hospital 1	Internal Medicine	Guillain-Barré syndrome	Peripheral venous catheter	4 days	Rehydratation	Serum	No
IVC27	17	Woman	Hospital 1	Internal Medicine	Breast necrosis	Peripheral venous catheter	5 days	Medicine	Serum	No
IVC28	31	Man	Hospital 1	Internal Medicine	Vein thrombosis+ leg infection (Diabetes)	Peripheral venous catheter	7 days	Medicine	Anti-inflammatory	No
URC1	52	Man	Hospital 2	Infectious diseases	Tuberculosis	Urinary catheter	8 days	Bedridden	Antibiotics	No
URC2	86	Woman	Private healthcare unit	General Surgery	Hip prothesis	Urinary catheter	10 days	Surgery	Antibiotics	No
URC3	40	Woman	Private healthcare unit	General Surgery	Gynecological problem	Urinary catheter	2 days	Laparoscopy	Antibiotics	No

Table 4. General characteristic of the studied patients (URC4-IAD4).

Patient	Age	Gender	Hospital	Admission department	Reason for admission	Medical device used	Duration *in situ*	Pathology that motivated the use of the device	Ongoing treatment	Symptoms before device removal
URC4	77	Woman	Hospital 1	Resuscitation	Severe asthma	Urinary catheter	8 days	Coma	Antibiotics	No
URC5 **IVC9**	59	Woman	Hospital 1	Resuscitation	Hypertrophic heart disease	Urinary catheter Femoral venous catheter	16 days 15 days	Coma	Antibiotics	No
URC6	96	Woman	Hospital 1	General Surgery	Extended 2nd degree burn	Urinary catheter	6 days	Bedridden	Antibiotics	No
URC7	20	Woman	Hospital 1	Resuscitation	Meningitis	Urinary catheter	4 days	Coma	Antibiotics antivirals	No
URC8	80	Woman	Hospital 1	Internal Medicine	Vein thrombosis	Urinary catheter	3 days	Bedridden	Anticoagulant	No
IAD1	31	Man	Hospital 1	General Surgery	Peritonitis (Gastric ulcer perforation)	Intra-abdominal blade	3 days	Surgery	Antibiotics	No
IAD2	40	Man	Hospital 1	General Surgery	Peritonitis (Gastric ulcer perforation)	Intra-abdominal blade	11 days	Surgery	Antibiotics	No
IAD3	22	Man	Hospital 1	General Surgery	Appendiceal peritonitis	Intra-abdominal blade	5 days	Surgery	Antibiotics	No
IAD4	63	Man	Hospital 1	General Surgery	Liver abscess	Intra-abdominal blade	10 days	Medicine	Serum	No

Appendix V

Table 1. Results of the microbial analysis of medical devices.

Medical device		Enumeration (CFU/mL)
IVC1	TSA	$1.31x10^3$
	NA	$1.33x10^3$
	EMB	$4.73x10^2$
	MSA	-
IVC2	TSA	$1.15x10^3$
	NA	$1.21x10^3$
	EMB	$1.33\ x10^3$
	MSA	$8.7\ x10^1$
IVC3	TSA	
	NA	$\geq 10^3$
	EMB	
	MSA	$1.13\ x10^2$
IVC4	TSA	$1.20x10^2$
	NA	$1.73x10^2$
	EMB	-
	MSA	-
IVC5	TSA	$5.33x10^1$
	NA	$8x10^1$
	EMB	-
	MSA	-
IVC6	TSA	$6x10^1$
	NA	$1.13x10^2$
	EMB	-
	MSA	-
IVC7	TSA	$7.33x10^1$
	NA	$1.20x10^2$
	EMB	$2.67x10^1$
	MSA	$6x10^1$
IVC8	TSA	$4.67x10^1$
	NA	$6.67x10^1$
	EMB	-
	MSA	$0.67x10^1$
IVC10	TSA	$1.26x10^3$
	NA	$1.11x10^3$
	EMB	$6.67x10^1$
	MSA	$3.3x10^1$
IVC11	TSA	$1.71x10^3$
	NA	$1.73x10^3$
	EMB	$8x10^1$
	MSA	$6.7x10^1$
IVC12	TSA	$\geq 10^3$
	NA	$1.9x10^3$
	EMB	$6x10^1$
	MSA	$9.3x10^1$

Table 2. Results of the microbial analysis of medical devices.

Medical device		**Enumeration (CFU/mL)**
IVC13	TSA	
	NA	No growth
	EMB	
	MSA	
IVC14	TSA	
	NA	No growth
	EMB	
	MSA	
IVC15	TSA	$2x10^1$
	NA	-
	EMB	-
	MSA	-
IVC16	TSA	$1.25x10^3$
	NA	$1.1x10^3$
	EMB	-
	MSA	$4.73x10^2$
IVC17	TSA	$1.35x10^3$
	NA	$1.24x10^3$
	EMB	-
	MSA	$1.67x10^2$
IVC18	TSA	$3.3x10^1$
	NA	-
	EMB	-
	MSA	-
IVC19	TSA	$3.3x10^1$
	NA	$5.3x10^1$
	EMB	-
	MSA	$2x10^1$
IVC20	TSA	$2x10^1$
	NA	$5.3x10^1$
	EMB	-
	MSA	-
IVC21	TSA	
	NA	No growth
	EMB	
	MSA	
IVC22	TSA	
	NA	No growth
	EMB	
	MSA	
IVC23	TSA	$7.3x10^1$
	NA	$1.07x10^2$
	EMB	-
	MSA	-

Table 3. Results of the microbial analysis of medical devices.

Medical device		**Enumeration (CFU/mL)**
IVC24	TSA	$2.7x10^1$
	NA	-
	EMB	-
	MSA	-
IVC25	TSA	
	NA	No growth
	EMB	
	MSA	
IVC26	TSA	$1.17x10^3$
	NA	$8.33x10^2$
	EMB	-
	MSA	$8x10^1$
IVC27	TSA	-
	NA	$4.67x10^1$
	EMB	-
	MSA	-
IVC28	TSA	
	NA	No growth
	EMB	
	MSA	
URC3	TSA	$2x10^2$
	NA	$3x10^2$
	EMB	$8.7x10^1$
	MSA	-
URC6	TSA	$\geq 10^3$
	NA	$\geq 10^3$
	EMB	$\geq 10^3$
	MSA	$1.4x10^3$
URC7	TSA	$\geq 10^3$
	NA	$\geq 10^3$
	EMB	$1.2x10^3$
	MSA	$7.33x10^2$
URC8	TSA	$\geq 10^3$
	NA	$1.83x10^3$
	EMB	$8x10^1$
	MSA	$5.31x10^1$
IAD1	TSA	$1.73x10^2$
	NA	$8x10^1$
	EMB	$3.3x10^1$
	MSA	$2x10^1$
IAD3	TSA	$1x10^1$
	NA	$1.20x10^2$
	EMB	-
	MSA	$3.3x10^1$

Appendix VI

Table 1. Strains identity and origin (EC1-EF25)**.**

Strain	Origin	Identity	Strain	Origin	Identity
EC1	IVC11	*Escherichia coli*	**KP4**	URC8	*Klebsiella pneumoniae*
EC2	URC6	*Escherichia coli*	**KP5**	URC8	*Klebsiella pneumoniae*
EC3	IVC12	*Escherichia coli*	**KP6**	IAD1	*Klebsiella pneumoniae*
EC4	URC6	*Escherichia coli*	**AB1**	IVC12	*Acinetobacter baumannii*
EC5	URC3	*Escherichia coli*	**AB2**	IVC3	*Acinetobacter baumannii*
EC6	IVC12	*Escherichia coli*	**AB3**	IVC11	*Acinetobacter baumannii*
EC7	IVC11	*Escherichia coli*	**AB4**	IVC2	*Acinetobacter baumannii*
EC8	IVC1	*Escherichia coli*	**AB5**	IVC12	*Acinetobacter baumannii*
EC9	IVC3	*Escherichia coli*	**AL**	IVC2	*Acinetobacter lwoffii*
EC10	IVC1	*Escherichia coli*	**EF1**	IVC5	*Enterococcus feacalis*
EC11	IVC2	*Escherichia coli*	**EF2**	IVC6	*Enterococcus feacalis*
EC12	URC8	*Escherichia coli*	**EF3**	IVC4	*Enterococcus feacalis*
EC13	IVC3	*Escherichia coli*	**EF4**	IVC4	*Enterococcus faecalis*
EC14	IVC10	*Escherichia coli*	**EF5**	IVC3	*Enterococcus faecalis*
EC15	IVC11	*Escherichia coli*	**EF6**	IVC3	*Enterococcus faecalis*
EC16	IVC10	*Escherichia coli*	**EF7**	URC7	*Enterococcus faecalis*
EC17	IVC7	*Escherichia coli*	**EF8**	IVC13	*Enterococcus faecalis*
EC18	IVC7	*Escherichia coli*	**EF9**	IVC5	*Enterococcus faecalis*
EC19	IVC7	*Escherichia coli*	**EF10**	IVC13	*Enterococcus faecalis*
EC20	IVC7	*Escherichia coli*	**EF11**	URC6	*Enterococcus faecalis*
EC21	IVC11	*Escherichia coli*	**EF12**	IVC10	*Enterococcus faecalis*
EC22	IVC11	*Escherichia coli*	**EF13**	IVC10	*Enterococcus faecalis*
EC23	IVC5	*Escherichia coli*	**EF14**	URC7	*Enterococcus faecalis*
EC24	IVC3	*Escherichia coli*	**EF15**	IVC8	*Enterococcus faecalis*
EC25	IVC4	*Escherichia coli*	**EF16**	IVC1	*Enterococcus faecalis*
EC26	IVC4	*Escherichia coli*	**EF17**	IVC1	*Enterococcus faecalis*
EC27	IVC6	*Escherichia coli*	**EF18**	URC3	*Enterococcus faecalis*
EC28	IVC6	*Escherichia coli*	**EF19**	URC3	*Enterococcus faecalis*
EC29	IVC10	*Escherichia coli*	**EF20**	URC6	*Enterococcus faecalis*
EC30	IAD1	*Escherichia coli*	**EF21**	URC3	*Enterococcus faecalis*
EC31	IAD1	*Escherichia coli*	**EF22**	URC3	*Enterococcus faecalis*
KP1	URC7	*Klebsiella pneumoniae*	**EF23**	URC3	*Enterococcus faecalis*
KP2	URC7	*Klebsiella pneumoniae*	**EF24**	URC3	*Enterococcus faecalis*
KP3	URC6	*Klebsiella pneumoniae*	**EF25**	URC7	*Enterococcus faecalis*

IVC: Intravenous catheter; **URC:** urinary catheter; **IAD:** intra-abdominal drain.

Table 2. Strains identity and origin (EF26-ST60)**.**

Strain	Origin	Identity	Strain	Origin	Identity
EF26	URC6	*Enterococcus faecalis*	**ST27**	IVC2	*Staphylococcus aureus*
EF27	URC7	*Enterococcus faecalis*	**ST28**	IVC2	Unidentified
EF28	URC8	*Enterococcus faecalis*	**ST29**	IVC3	Unidentified
EF29	URC8	*Enterococcus faecalis*	**ST30**	IVC6	Unidentified
EF30	URC8	*Enterococcus faecalis*	**ST31**	IVC3	*Staphylococcus haemolyticus*
EF31	URC9	*Enterococcus faecalis*	**ST32**	IVC1	*Staphylococcus haemolyticus*
EF32	URC3	*Enterococcus faecalis*	**ST33**	IVC2	*Staphylococcus haemolyticus*
EF33	IVC1	*Enterococcus faecalis*	**ST34**	IVC12	*Staphylococcus aureus*
ST1	IVC3	*Staphylococcus aureus*	**ST35**	IVC7	*Staphylococcus aureus*
ST2	IVC10	*Staphylococcus aureus*	**ST36**	IVC7	Unidentified
ST3	IVC10	*Staphylococcus aureus*	**ST37**	IVC10	Unidentified
ST4	IVC10	*Staphylococcus cohnii*	**ST38**	IVC10	Unidentified
ST5	IVC2	Unidentified	**ST39**	IAD1	Unidentified
ST6	IVC10	Unidentified	**ST40**	IAD1	*Staphylococcus aureus*
ST7	IVC10	Unidentified	**ST41**	IVC11	Unidentified
ST8	IVC12	*Staphylococcus aureus*	**ST42**	IAD1	*Staphylococcus aureus*
ST9	IVC12	*Staphylococcus aureus*	**ST43**	IAD1	*Staphylococcus aureus*
ST10	IVC12	*Staphylococcus aureus*	**ST44**	IAD1	*Staphylococcus aureus*
ST11	IVC12	*Staphylococcus aureus*	**ST45**	IVC11	Unidentified
ST12	IVC2	*Staphylococcus aureus*	**ST46**	IVC1	Unidentified
ST13	IAD1	*Staphylococcus aureus*	**ST47**	IVC1	Unidentified
ST14	IAD1	*Staphylococcus aureus*	**ST48**	IVC23	Unidentified
ST15	IVC1	*Staphylococcus aureus*	**ST49**	IVC23	Unidentified
ST16	URC6	*Staphylococcus aureus*	**ST50**	IVC5	Unidentified
ST17	URC6	*Staphylococcus aureus*	**ST51**	IVC19	Unidentified
ST18	IVC3	*Staphylococcus aureus*	**ST52**	IVC16	Unidentified
ST19	URC6	*Staphylococcus aureus*	**ST53**	IVC23	Unidentified
ST20	URC8	*Staphylococcus aureus*	**ST54**	IVC7	Unidentified
ST21	IVC11	*Staphylococcus aureus*	**ST55**	URC7	Unidentified
ST22	IVC11	Unidentified	**ST56**	IVC23	Unidentified
ST23	IVC11	Unidentified	**ST57**	IVC20	Unidentified
ST24	IVC5	Unidentified	**ST58**	IAD3	Unidentified
ST25	IVC5	Unidentified	**ST59**	IVC5	Unidentified
ST26	IVC11	Unidentified	**ST60**	IVC16	Unidentified

IVC: Intravenous catheter; **URC:** urinary catheter; **IAD:** intra-abdominal drain.

Table 3. Strains identity and origin (ST61-ST128)**.**

Strain	Origin	Identity	Strain	Origin	Identity
ST61	IVC18	Unidentified	**ST95**	IVC18	Unidentified
ST62	IVC26	Unidentified	**ST96**	IVC18	Unidentified
ST63	IVC26	Unidentified	**ST97**	IVC26	Unidentified
ST64	IVC23	Unidentified	**ST98**	IVC26	Unidentified
ST65	IVC16	Unidentified	**ST99**	IVC19	Unidentified
ST66	IVC19	Unidentified	**ST100**	IVC16	Unidentified
ST67	IVC23	Unidentified	**ST101**	IVC16	Unidentified
ST68	IAD3	Unidentified	**ST102**	IVC20	Unidentified
ST69	IVC27	Unidentified	**ST103**	IVC18	Unidentified
ST70	IAD3	Unidentified	**ST104**	IVC27	Unidentified
ST71	IAD3	Unidentified	**ST105**	IVC8	Unidentified
ST72	IVC26	Unidentified	**ST106**	IVC20	Unidentified
ST73	IVC17	Unidentified	**ST107**	IVC19	Unidentified
ST74	IVC17	Unidentified	**ST108**	IVC19	Unidentified
ST75	IVC17	Unidentified	**ST109**	IVC23	Unidentified
ST76	IVC16	Unidentified	**ST110**	IVC4	Unidentified
ST77	IVC17	Unidentified	**ST111**	IAD3	Unidentified
ST78	IVC17	Unidentified	**ST112**	IVC15	Unidentified
ST79	IVC15	Unidentified	**ST113**	IVC6	Unidentified
ST80	IVC16	Unidentified	**ST114**	IVC19	Unidentified
ST81	IVC15	Unidentified	**ST115**	IVC20	Unidentified
ST82	IVC18	Unidentified	**ST116**	IVC24	Unidentified
ST83	IVC15	Unidentified	**ST117**	IVC24	Unidentified
ST84	IVC17	Unidentified	**ST118**	IVC24	Unidentified
ST85	IVC18	Unidentified	**ST119**	IVC19	Unidentified
ST86	IVC17	Unidentified	**ST120**	IVC19	Unidentified
ST87	IVC4	Unidentified	**ST121**	IVC19	Unidentified
ST88	IAD3	Unidentified	**ST122**	KT8	Unidentified
ST89	IAD3	Unidentified	**ST123**	IVC20	Unidentified
ST90	IVC4	Unidentified	**ST124**	IVC20	Unidentified
ST91	IVC7	Unidentified	**ST125**	IVC26	Unidentified
ST92	IVC23	Unidentified	**ST126**	IVC19	Unidentified
ST93	IVC16	Unidentified	**ST127**	IVC6	Unidentified
ST94	URC7	Unidentified	**ST128**	IVC19	Unidentified

IVC: Intravenous catheter; **URC:** urinary catheter; **IAD:** intra-abdominal drain.

Table 4. Strains identity and origin (ST129-ST143)**.**

Strain	Origin	Identity
ST129	IVC16	Unidentified
ST130	IAD3	Unidentified
ST131	IVC19	Unidentified
ST132	IVC24	Unidentified
ST133	IVC17	Unidentified
ST134	IVC17	Unidentified
ST135	IVC16	Unidentified
ST136	IVC18	Unidentified
ST137	IVC15	Unidentified
ST138	IVC26	Unidentified
ST139	IVC26	Unidentified
ST140	IAD3	Unidentified
ST141	IVC27	Unidentified
ST142	IVC27	Unidentified
ST143	IVC24	Unidentified

IVC: Intravenous catheter; **URC:** urinary catheter; **IAD:** intra-abdominal drain.

Appendix VII

Table 1A. Biofilm formation of *Enterobacteriaceae* strains (EC1-EC29) isolated from medical devices using TCP and Congo red agar assays. The values are the means of three independent experiments.

Identity	Biofilm formation/TCP assay (OD630nm)		Slime production
Escherichia coli EC1	0.026±0.010	Non adherent	Non slime producer
Escherichia coli EC2	0.376±0.022	Strongly adherent	Non slime producer
Escherichia coli EC3	0.028±0.009	Non adherent	Non slime producer
Escherichia coli EC4	0.101±0.019	Moderately adherent	Non slime producer
Escherichia coli EC5	0.259±0.006	Strongly adherent	Non slime producer
Escherichia coli EC6	0.271±0.015	Strongly adherent	Non slime producer
Escherichia coli EC7	0.266±0.022	Strongly adherent	Non slime producer
Escherichia coli EC8	0.292±0.024	Strongly adherent	Non slime producer
Escherichia coli EC9	0.222±0.012	Strongly adherent	Non slime producer
Escherichia coli EC10	0.284±0.018	Strongly adherent	Non slime producer
Escherichia coli EC11	0.027±0.011	Non adherent	Non slime producer
Escherichia coli EC12	0.068±0.022	Moderately adherent	Non slime producer
Escherichia coli EC13	0.243±0.015	Strongly adherent	Non slime producer
Escherichia coli EC14	0.051±0.007	Weakly adherent	Non slime producer
Escherichia coli EC15	0.044±0.019	Weakly adherent	Non slime producer
Escherichia coli EC16	0.094±0.008	Moderately adherent	Non slime producer
Escherichia coli EC17	0.141±0.017	Strongly adherent	Non slime producer
Escherichia coli EC18	0.096±0.011	Moderately adherent	Non slime producer
Escherichia coli EC19	0.047±0.034	Weakly adherent	Non slime producer
Escherichia coli EC20	0.138±0.021	Strongly adherent	Non slime producer
Escherichia coli EC21	0.04±0.023	Weakly adherent	Non slime producer
Escherichia coli EC22	0.122±0.003	Strongly adherent	Non slime producer
Escherichia coli EC23	0.105±0.007	Moderately adherent	Non slime producer
Escherichia coli EC24	0.101±0.029	Moderately adherent	Non slime producer
Escherichia coli EC25	0.042±0.015	Weakly adherent	Non slime producer
Escherichia coli EC26	0.039±0.006	Weakly adherent	Non slime producer
Escherichia coli EC27	0.024±0.003	Non adherent	Non slime producer
Escherichia coli EC28	0.023±0.011	Non adherent	Non slime producer
Escherichia coli EC29	0.052±0.008	Weakly adherent	Non slime producer

Table 1B. Biofilm formation of *Enterobacteriaceae* strains (EC30-KP6) isolated from medical devices using TCP and Congo red agar assays. The values are the means of three independent experiments.

Identity	Biofilm formation/TCP assay (OD630nm)		Slime production
Escherichia coli EC30	0.277±0.016	Strongly adherent	Non slime producer
Escherichia coli EC31	0.027±0.017	Non adherent	Non slime producer
Klebsiella pneumoniae KP1	0.292±0.041	Strongly adherent	Non slime producer
Klebsiella pneumoniae KP2	0.254±0.022	Strongly adherent	Non slime producer
Klebsiella pneumoniae KP3	0.121±0.031	Strongly adherent	Non slime producer
Klebsiella pneumoniae KP4	0.187±0.011	Strongly adherent	Non slime producer
Klebsiella pneumoniae KP5	0.074±0.031	Moderately adherent	Non slime producer
Klebsiella pneumoniae KP6	0.150±0.016	Strongly adherent	Non slime producer

Table 2. Biofilm formation of *Acinetobacter* spp. strains isolated from medical devices using TCP and Congo red agar assays. The values are the means of three independent experiments.

Identity	Biofilm formation/TCP assay (OD630nm)		Slime production
Acinetobacter baumannii AB1	0.242±0.020	Strongly adherent	Non slime producer
Acinetobacter baumannii AB2	0.88±0.013	Moderately adherent	Non slime producer
Acinetobacter baumanniiAB3	0.057±0.025	Weakly adherent	Non slime producer
Acinetobacter lwoffii AL	0.322±0.027	Strongly adherent	Non slime producer
Acinetobacter baumannii AB4	0.059±0.015	Weakly adherent	Non slime producer
Acinetobacter baumannii AB5	0.021±0.013	Non adherent	Non slime producer

Table 3A. Biofilm formation of *Staphylococcus* spp. strains (ST1-ST30) isolated from medical devices using TCP and Congo red agar assays. The values are the means of three independent experiments.

Identity	Biofilm formation/TCP assay (OD630nm)		Slime production
***Staphylococcus aureus* ST1**	0.122±0.022	Strongly adherent	Non slime producer
***Staphylococcus aureus* ST2**	0.424±0.037	Strongly adherent	Strong slime producer
***Staphylococcus aureus* ST3**	0.406±0.040	Strongly adherent	Strong slime producer
***Staphylococcus cohnii* ST4**	0.182±0.016	Strongly adherent	Non slime producer
***Staphylococcus* spp.ST5**	0.351±0.045	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST6**	0.025±0.008	Non adherent	Strong slime producer
***Staphylococcus* spp.ST7**	0.284±0.024	Strongly adherent	Weak slime producer
***Staphylococcus aureus* ST8**	0.345±0.031	Strongly adherent	Strong slime producer
***Staphylococcus aureus* ST9**	0.366±0.022	Strongly adherent	Strong slime producer
***Staphylococcus aureus* ST10**	0.357±0.037	Strongly adherent	Strong slime producer
***Staphylococcus aureus* ST11**	0.246±0.026	Strongly adherent	Strong slime producer
***Staphylococcus aureus* ST12**	0.294±0.033	Strongly adherent	Non slime producer
***Staphylococcus aureus* ST13**	0.253±0.009	Strongly adherent	Strong slime producer
***Staphylococcus aureus* ST14**	0.343±0.039	Strongly adherent	Strong slime producer
***Staphylococcus aureus* ST15**	0.276±0.031	Strongly adherent	Weak slime producer
***Staphylococcus aureus* ST16**	0.512±0.039	Strongly adherent	Weak slime producer
***Staphylococcus aureus* ST17**	0.662±0.004	Strongly adherent	Weak slime producer
***Staphylococcus aureus* ST18**	0.404±0.037	Strongly adherent	Non slime producer
***Staphylococcus aureus* ST19**	0.567±0.028	Strongly adherent	Non slime producer
***Staphylococcus aureus* ST20**	0.407±0.024	Strongly adherent	Non slime producer
***Staphylococcus aureus* ST21**	0.617±0.004	Strongly adherent	Non slime producer
***Staphylococcus* spp.ST22**	0.118±0.011	Moderately adherent	Non slime producer
***Staphylococcus* spp.ST23**	0.08±0.015	Moderately adherent	Non slime producer
***Staphylococcus* spp.ST24**	0.319±0.046	Strongly adherent	Non slime producer
***Staphylococcus* spp.ST25**	0.266±0.019	Strongly adherent	Non slime producer
***Staphylococcus* spp.ST26**	0.071±0.012	Moderately adherent	Non slime producer
***Staphylococcus aureus* ST27**	0.637±0.023	Strongly adherent	Strong slime producer
***Staphylococcus* spp.ST28**	0.521±0.049	Strongly adherent	Non slime producer
***Staphylococcus* spp.ST29**	0.564±0.008	Strongly adherent	Non slime producer
***Staphylococcus* spp.ST30**	0.375±0.017	Strongly adherent	Strong slime producer

Table 3B. Biofilm formation of *Staphylococcus* spp. strains (ST31-ST60) isolated from medical devices using TCP and Congo red agar assays. The values are the means of three independent experiments.

Identity	Biofilm formation/TCP assay (OD630nm)		Slime production
***Staphylococcus haemolyticus* ST31**	0.354±0.039	Strongly adherent	Weak slime producer
***Staphylococcus haemolyticus* ST32**	0.325±0.005	Strongly adherent	Weak slime producer
***Staphylococcus haemolyticus* ST33**	0.280±0.018	Strongly adherent	Non slime producer
***Staphylococcus aureus* ST34**	0.341±0.037	Strongly adherent	Strong slime producer
***Staphylococcus aureus* ST35**	0.350±0.017	Strongly adherent	Strong slime producer
***Staphylococcus* spp. ST36**	0.240±0.036	Strongly adherent	Strong slime producer
***Staphylococcus* spp. ST37**	0.237±0.028	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST38**	0.328±0.033	Strongly adherent	Strong slime producer
***Staphylococcus* spp. ST39**	0.216±0.030	Strongly adherent	Strong slime producer
***Staphylococcus aureus* ST40**	0.186±0.021	Strongly adherent	Strong slime producer
***Staphylococcus* spp. ST41**	0.157±0.041	Strongly adherent	Non slime producer
***Staphylococcus aureus* ST42**	0.297±0.031	Strongly adherent	Strong slime producer
***Staphylococcus aureus* ST43**	0.277±0.012	Strongly adherent	Strong slime producer
***Staphylococcus aureus* ST44**	0.299±0.011	Strongly adherent	Strong slime producer
***Staphylococcus* spp. ST45**	0.097±0.010	Moderately adherent	Strong slime producer
***Staphylococcus* spp. ST46**	0.053±0.012	Weakly adherent	Non slime producer
***Staphylococcus* spp. ST47**	0.111±0.008	Moderately adherent	Strong slime producer
***Staphylococcus* spp. ST48**	0.684±0.032	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST49**	0.698±0.045	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST50**	1.04±0.022	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST51**	1.00±0.036	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST52**	1.49±0.037	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST53**	0.865±0.037	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST54**	0.620±0.035	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST55**	1.16±0.012	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST56**	0.681±0.016	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST57**	0.861±0.034	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST58**	1.17±0.024	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST59**	1.21±0.024	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST60**	1.75±0.027	Strongly adherent	Weak slime producer

Table 3C. Biofilm formation of *Staphylococcus* spp. strains (ST61-ST90) isolated from medical devices using TCP and Congo red agar assays. The values are the means of three independent experiments.

Identity	Biofilm formation/TCP assay (OD630nm)		Slime production
***Staphylococcus* spp. ST61**	1.145±0.031	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST62**	1.099±0.019	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST63**	0.992±0.004	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST64**	0.611±0.034	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST65**	1.647±0.032	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST66**	1.637±0.027	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST67**	0.852±0.037	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST68**	0.273±0.016	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST69**	1.071±0.021	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST70**	0.05±0.035	Weakly adherent	Weak slime producer
***Staphylococcus* spp. ST71**	0.224±0.037	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST72**	0.987±0.037	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST73**	0.261±0.027	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST74**	0.328±0.017	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST75**	0.255±0.032	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST76**	0.752±0.028	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST77**	0.539±0.023	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST78**	0.603±0.018	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST79**	0.662±0.022	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST80**	0.655±0.032	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST81**	1.111±0.036	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST82**	0.193±0.015	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST83**	0.237±0.020	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST84**	0.348±0.004	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST85**	0.497±0.009	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST86**	0.366±0.026	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST87**	0.558±0.030	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST88**	0.466±0.038	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST89**	0.183±0.005	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST90**	0.291±0.025	Strongly adherent	Non slime producer

Table 3D. Biofilm formation of *Staphylococcus* spp. strains (ST91-ST120) isolated from medical devices using TCP and Congo red agar assays. The values are the means of three independent experiments.

Identity	Biofilm formation/TCP assay (OD630nm)		Slime production
***Staphylococcus* spp. ST91**	0.404±0.017	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST92**	0.179±0.031	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST93**	0.755±0.019	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST94**	0.195±0.038	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST95**	0.306±0.030	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST96**	0.219±0.021	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST97**	0.546±0.030	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST98**	0.497±0.011	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST99**	0.609±0.027	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST100**	1.094±0.008	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST101**	0.638±0.037	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST102**	1.19±0.013	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST103**	1.367±0.051	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST104**	0.476±0.023	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST105**	0977±0.005	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST106**	0.488±0.023	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST107**	0.812±0.022	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST108**	0.762±0.038	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST109**	0.512±0.021	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST110**	0.645±0.034	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST111**	0.773±0.039	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST112**	0.871±0.019	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST113**	0.518±0.024	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST114**	0.869±0.028	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST115**	0.891±0.032	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST116**	1.030±0.033	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST117**	0.817±0.030	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST118**	0.859±0.027	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST119**	0.863±0.018	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST120**	0.695±0.007	Strongly adherent	Non slime producer

Table 3E. Biofilm formation of *Staphylococcus* spp. strains (ST121-ST143) isolated from medical devices using TCP and Congo red agar assays. The values are the means of three independent experiments.

Identity	Biofilm formation/TCP assay (OD630nm)		Slime production
***Staphylococcus* spp. ST121**	0.186±0.018	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST122**	0.329±0.052	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST123**	0.321±0.050	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST124**	0.871±0.028	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST125**	0.549±0.028	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST126**	0.668±0.016	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST127**	0.655±0.014	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST128**	0.501±0.038	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST129**	0.716±0.038	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST130**	0.624±0.037	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST131**	1.194±0.010	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST132**	0.427±0.019	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST133**	0.892±0.022	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST134**	0.866±0.047	Strongly adherent	Non slime producer
***Staphylococcus* spp. ST135**	0.480±0.044	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST136**	1.274±0.013	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST137**	0.393±0.028	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST138**	0.585±0.033	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST139**	0.477±0.028	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST140**	0.843±0.035	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST141**	0.499±0.003	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST142**	0.456±0.020	Strongly adherent	Weak slime producer
***Staphylococcus* spp. ST143**	0.325±0.011	Strongly adherent	Weak slime producer

Table 4A. Biofilm formation of *Enterococcus faecalis* strains (EF1-EF30) isolated from medical devices using TCP and Congo red agar assays. The values are the means of three independent experiments.

Identity	Biofilm formation/TCP assay (OD630nm)		Slime production
***Enteroococcus faecalis* EF1**	0.198±0.020	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF2**	0.368±0.031	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF3**	0.371±0.033	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF4**	0.171±0.009	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF5**	0.225±0.026	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF6**	0.244±0.030	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF7**	0.367±0.027	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF8**	0.348±0.022	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF9**	0.274±0.028	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF10**	0.244±0.038	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF11**	0.163±0.017	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF12**	0.183±0.008	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF13**	0.215±0.008	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF14**	0.135±0.029	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF15**	0.206±0.022	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF16**	0.256±0.026	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF17**	0.270±0.033	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF18**	1.528±0.016	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF19**	1.235±0.008	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF20**	1.191±0.012	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF21**	1.225±0.035	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF22**	0.679±0.022	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF23**	0.707±0.018	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF24**	0.584±0.026	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF25**	0.466±0.036	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF26**	0.697±0.028	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF27**	0.707±0.039	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF28**	1.428±0.026	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF29**	1.555±0.024	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF30**	0.698±0.033	Strongly adherent	Non slime producer

Table 4B. Biofilm formation of *Enterococcus faecalis* strains (EF31-EF33) isolated from medical devices using TCP and Congo red agar assays. The values are the means of three independent experiments.

Identity	Biofilm formation/TCP assay (OD630nm)		Slime production
***Enteroococcus faecalis* EF31**	0.693±0.036	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF32**	0.638±0.011	Strongly adherent	Non slime producer
***Enteroococcus faecalis* EF33**	0.227±0.005	Strongly adherent	Non slime producer

Appendix VIII

Table 1. Average diameters of the zones of inhibition (mm) obtained for the antibiotics tested with regard to the strains of *Enterobacteriaceae* isolates.

Strain	AMC (20+10)	CTX (30)	CAZ (30)	FEP (30)	FOX (30)	IPM (10)	TET (30)	NA (30)	CIP (5)	GEN (10)	CO-TRI (1.25+23.75)
EC1	20 (S)	33 (S)	31 (S)	30 (S)	27 (S)	31 (S)	06 (R)	06 (R)	27 (S)	25 (S)	30 (S)
EC2	22 (S)	31 (S)	28 (S)	38 (S)	25 (S)	31 (S)	06 (R)	06 (R)	28 (S)	16 (I)	40 (S)
EC3	20 (S)	33 (S)	30 (S)	28 (S)	30 (S)	27 (S)	23 (S)	28 (S)	36 (S)	25 (S)	06 (R)
EC4	22 (S)	29 (S)	28 (S)	30 (S)	22 (S)	35 (S)	20 (S)	06 (R)	26 (S)	22 (S)	35 (S)
EC5	24 (S)	28 (S)	26 (S)	34 (S)	24 (S)	31 (S)	26 (S)	06 (R)	25 (I)	23 (S)	34 (S)
EC6	19 (S)	26 (S)	24 (S)	32 (S)	24 (S)	36 (S)	22 (S)	06 (R)	27 (S)	24 (S)	32 (S)
EC7	20 (S)	32 (S)	30 (S)	29 (S)	26 (S)	30 (S)	20 (S)	06 (R)	26 (S)	24 (S)	30 (S)
EC8	27 (S)	34 (S)	28 (S)	32 (S)	24 (S)	27 (S)	13 (I)	06 (R)	28 (S)	24 (S)	32 (S)
EC9	20 (S)	27 (S)	30 (S)	38 (S)	27 (S)	27 (S)	06 (R)	06 (R)	27 (S)	25 (S)	35 (S)
EC10	18 (R)	30 (S)	25 (S)	33 (S)	21 (S)	28 (S)	23 (S)	07 (R)	27 (S)	24 (S)	32 (S)
EC11	06 (R)	31 (S)	30 (S)	29 (S)	27 (S)	28 (S)	21 (S)	26(S)	34 (S)	21 (S)	06 (R)
EC12	19 (S)	31 (S)	28 (S)	28 (S)	26 (S)	29 (S)	21 (S)	06 (R)	28 (S)	24 (S)	33 (S)
EC13	19 (S)	30 (S)	27 (S)	29 (S)	24 (S)	29 (S)	20 (S)	24 (S)	24 (I)	24 (S)	30 (S)
EC14	06 (R)	32 (S)	30 (S)	30 (S)	28 (S)	30 (S)	22 (S)	28 (S)	32 (S)	21 (S)	06 (R)
EC15	18 (R)	32 (S)	30 (S)	32 (S)	25 (S)	30 (S)	06 (R)	26 (S)	30 (S)	24 (S)	30 (S)
EC16	20 (S)	35 (S)	26 (S)	35 (S)	27 (S)	30(S)	06 (R)	06 (R)	26 (S)	25 (S)	35 (S)
EC17	20 (S)	35 (S)	30 (S)	33 (S)	27 (S)	29 (S)	06 (R)	06 (R)	30 (S)	27 (S)	33 (S)
EC18	20 (S)	33 (S)	30 (S)	32 (S)	28 (S)	29 (S)	30 (S)	06 (R)	25 (I)	29 (S)	31 (S)
EC19	19 (S)	31 (S)	30 (S)	34 (S)	27 (S)	29 (S)	06 (R)	08 (R)	29 (S)	27 (S)	32 (S)
EC20	21 (S)	32 (S)	27 (S)	32 (S)	27 (S)	27 (S)	27 (S)	06 (R)	25 (I)	24 (S)	33 (S)
EC21	20 (S)	35 (S)	31 (S)	36 (S)	27 (S)	30 (S)	06 (R)	06 (R)	31 (S)	30 (S)	32 (S)
EC22	18 (R)	31 (S)	30 S)	36 (S)	25 (S)	29 (S)	32 (S)	06 (R)	30 (S)	27 (S)	35 (S)
EC23	28 (S)	34 (S)	30 (S)	30 (S)	26 (S)	30 (S)	34 (S)	06 (R)	25 (I)	23 (S)	30 (S)
EC24	21 (S)	33 (S)	30 (S)	39 (S)	27 (S)	29 (S)	06 (R)	08 (R)	32 (S)	32 (S)	36 (S)
EC25	22 (S)	35 (S)	32 (S)	41 (S)	26 (S)	32 (S)	06 (R)	06 (R)	35 (S)	32 (S)	35 (S)
EC26	18 (R)	39 (S)	36 (S)	42 (S)	32 (S)	34 (S)	30 (S)	06 (R)	29 (S)	26 (S)	33 (S)
EC27	20 (S)	33 (S)	31 (S)	40 (S)	26 (S)	31 (S)	07 (R)	06 (R)	29 (S)	29 (S)	36 (S)
EC28	16 (R)	30 (S)	27 (S)	29 (S)	27 (S)	28 (S)	20 (S)	26 (S)	32 (S)	22 (S)	06 (R)
EC29	24 (S)	36 (S)	34 (S)	30 (S)	28 (S)	25 (S)	22 (S)	28 (S)	26 (S)	29 (S)	36 (S)
EC30	21 (S)	26 (S)	29 (S)	33 (S)	26 (S)	24 (S)	20 (S)	06 (R)	24 (I)	24 (S)	33 (S)
EC31	20 (S)	36 (S)	34 (S)	34 (S)	27 (S)	29 (S)	06 (R)	08 (R)	27 (S)	26 (S)	31 (S)
KP1	18 (R)	27 (S)	27 (S)	11 (R)	23 (S)	30 (S)	08 (R)	24 (S)	25 (I)	11 (R)	26 (S)
KP2	17 (R)	11 (R)	13 (R)	14 (R)	24 (S)	25 (S)	08 (R)	24 (S)	27 (S)	14 (I)	27 (S)
KP3	10 (R)	10 (R)	15 (R)	13 (R)	29 (S)	32 (S)	08 (R)	25 (S)	27 (S)	10 (R)	22 (S)
KP4	06 (R)	11 (R)	18 (R)	11 (R)	27 (S)	28 (S)	08 (R)	28 (S)	26 (S)	23 (S)	28 (S)
KP5	15 (R)	12 (R)	16 (R)	13 (R)	25 (S)	29 (S)	07 (R)	24 (S)	25 (I)	15(I)	23 (S)
KP6	06 (R)	10 (R)	16 (R)	13 (R)	29 (S)	22 (S)	22 (S)	28 (S)	26 (S)	24(S)	24 (S)

S: Sensitive, **R:** resistant, **I:** Intermediate.

Table 2. Average diameters of the zones of inhibition (mm) obtained for the antibiotics tested with regard to the strains of *Acinetobacter* spp. isolates.

Strain	Antibiotic (concentration µg)							
	CTX (30)	CAZ (30)	FEP (30)	IPM (10)	TET (30)	CIP (5)	GEN (10)	CO-TRI (1.25+23.75)
AB1	06 (R)	07 (R)	06 (R)	10 (R)	06 (R)	06 (R)	20 (S)	06 (R)
AB2	06 (R)	07 (R)	07 (R)	08 (R)	06 (R)	07 (R)	24 (S)	06 (R)
AB3	06 (R)	08 (R)	06 (R)	09 (R)	06 (R)	06 (R)	18 (S)	06 (R)
AB4	06 (R)	08 (R)	06 (R)	10 (R)	08 (R)	06 (R)	23 (S)	06 (R)
AB5	06 (R)	06 (R)	06 (R)	10 (R)	06 (R)	06 (R)	26 (S)	06 (R)
AL	24 (S)	28 (S)	10 (R)	08 (R)	08 (R)	09 (R)	24 (S)	06 (R)

S: Sensitive, **R:** resistant, **I:** Intermediate.

Table 3A. Average diameters of the zones of inhibition (mm) obtained for the antibiotics tested with regard to the strains of *Staphylococcus* spp. isolates (ST1-ST37).

Strain	Antibiotic (concentration µg)								
	FOX (30)	ERY (15)	GEN (10)	TET (30)	CIP (5)	RIF (5)	TOB (10)	CO-TRI (1.25+23.75)	CFT (30)
ST1	30 (S)	27 (S)	25 (S)	27 (S)	28 (S)	33 (S)	25 (S)	29 (S)	35 (S)
ST2	27 (S)	23 (S)	24 (S)	23 (S)	23 (S)	29 (S)	25 (S)	31 (S)	33(S)
ST3	28 (S)	25 (S)	26 (S)	26 (S)	26 (S)	28 (S)	24 (S)	32 (S)	35 (S)
ST4	23 (S)	07 (R)	25 (S)	08 (R)	25 (S)	34 (S)	35 (S)	21 (S)	/
ST5	28 (S)	10 (R)	33 (S)	08 (R)	35 (S)	40 (S)	34 (S)	30 (S)	/
ST6	29 (S)	07 (R)	29 (S)	18 (R)	31 (S)	40 (S)	30 (S)	22 (S)	/
ST7	20 (R)	15 (R)	40 (S)	08 (R)	40 (S)	41 (S)	36 (S)	37 (S)	/
ST8	25 (S)	06 (R)	12 (R)	08 (R)	09 (R)	30 (S)	24 (S)	30 (S)	32 (S)
ST9	28 (S)	24 (S)	25 (S)	21 (I)	25 (S)	29 (S)	23 (S)	30 (S)	33 (S)
ST10	25 (S)	25 (S)	22 (S)	22 (S)	25 (S)	28 (S)	23 (S)	31 (S)	29 (S)
ST11	28 (S)	27 (S)	25 (S)	27 (S)	27 (S)	27 (S)	21 (S)	27 (S)	34 (S)
ST12	34 (S)	29 (S)	40 (S)	34 (S)	30 (S)	40 (S)	39 (S)	43 (S)	41 (S)
ST13	29 (S)	25 (S)	21 (S)	24 (S)	26 (S)	33 (S)	24 (S)	30 (S)	32 (S)
ST14	24 (S)	20 (I)	23 (S)	24 (S)	25 (S)	29 (S)	26 (S)	28 (S)	35 (S)
ST15	34 (S)	25 (S)	23 (S)	25 (S)	25 (S)	33 (S)	23 (S)	33 (S)	27 (S)
ST16	27 (S)	25 (S)	22 (S)	25 (S)	25 (S)	27 (S)	24 (S)	31 (S)	32 (S)
ST17	30 (S)	25 (S)	21 (S)	26 (S)	26 (S)	29 (S)	22 (S)	30 (S)	30 (S)
ST18	30 (S)	26 (S)	21 (S)	27 (S)	27 (S)	30 (S)	22 (S)	31 (S)	35 (S)
ST19	27 (S)	26 (S)	23 (S)	25 (S)	26 (S)	33 (S)	24 (S)	25 (S)	30 (S)
ST20	26 (S)	27 (S)	24 (S)	26 (S)	25 (S)	35 (S)	23 (S)	26 (S)	27 (S)
ST21	27 (S)	26 (S)	24 (S)	26 (S)	26 (S)	33 (S)	23 (S)	27 (S)	32 (S)
ST22	30 (S)	26 (S)	07 (R)	27 (S)	30 (S)	26 (S)	07 (R)	28 (S)	/
ST23	33 (S)	06 (R)	06 (R)	27 (S)	33 (S)	27 (S)	07 (R)	07 (R)	/
ST24	34 (S)	15 (R)	32 (S)	28 (S)	32 (S)	33 (S)	32 (S)	33 (S)	/
ST25	32 (S)	07 (R)	24 (S)	30 (S)	28 (S)	34 (S)	14 (R)	06 (R)	/
ST26	28 (S)	12 (R)	35 (S)	29 (S)	35 (S)	24 (I)	26 (S)	27 (S)	/
ST27	29 (S)	25 (S)	23 (S)	30 (S)	27 (S)	28 (S)	25 (S)	26 (S)	30 (S)
ST28	29 (S)	14 (R)	29 (S)	25 (S)	32 (S)	35 (S)	29 (S)	16 (I)	/
ST29	29 (S)	26 (S)	30 (S)	20 (I)	32 (S)	40 (S)	30 (S)	21 (S)	/
ST30	35 (S)	24 (S)	25 (S)	19 (I)	30 (S)	45 (S)	24 (S)	24 (S)	/
ST31	15 (R)	19 (I)	16 (R)	09 (R)	19 (R)	40 (S)	30 (S)	06 (R)	/
ST32	16 (R)	16 (R)	15 (R)	18 (R)	16 (R)	27 (S)	09 (R)	07 (R)	/
ST33	28 (S)	29 (S)	25 (S)	29 (S)	26 (S)	39 (S)	27 (S)	22 (S)	/
ST34	28 (S)	32 (S)	28 (S)	29 (S)	28 (S)	27 (S)	30 (S)	27 (S)	36 (S)
ST35	27 (S)	29 (S)	30 (S)	33 (S)	30 (S)	29 (S)	26 (S)	28 (S)	35 (S)
ST36	29 (S)	34 (S)	29 (S)	28 (S)	36 (S)	40 (S)	32 (S)	10 (R)	/
ST37	34 (S)	09 (R)	06 (R)	20 (I)	06 (R)	35 (S)	07 (R)	06 (R)	/

S: Sensitive, **R:** resistant, **I:** Intermediate. **Blue box:***S. aureus*.

Table 3B. Average diameters of the zones of inhibition (mm) obtained for the antibiotics tested with regard to the strains of *Staphylococcus* spp. isolates (ST38-ST75).

Strain	Antibiotic (concentration µg)								
	FOX (30)	ERY (15)	GEN (10)	TET (30)	CIP (5)	RIF (5)	TOB (10)	CO-TRI (1.25+23.75)	CFT (30)
ST38	11 (R)	16 (R)	06 (R)	24 (S)	06 (R)	06 (R)	41 (S)	06 (R)	/
ST39	15 (R)	06 (R)	09 (R)	24 (S)	06 (R)	15 (R)	08 (R)	21 (S)	/
ST40	28 (S)	26 (S)	24 (S)	24 (S)	24 (S)	32 (S)	24 (S)	29 (S)	29 (S)
ST41	29 (S)	23 (S)	40 (S)	28 (S)	33 (S)	29 (S)	36 (S)	35 (S)	/
ST42	27 (S)	29 (S)	25 (S)	26 (S)	29 (S)	25 (I)	25 (S)	25 (S)	30 (S)
ST43	25 (S)	32 (S)	27 (S)	29 (S)	29 (S)	35 (S)	25 (S)	27 (S)	30 (S)
ST44	25 (S)	29 (S)	26 (S)	29 (S)	29 (S)	27 (S)	26 (S)	28 (S)	35 (S)
ST45	29 (S)	30 (S)	30 (S)	30 (S)	35 (S)	33 (S)	29 (S)	32 (S)	/
ST46	34 (S)	24 (S)	22 (S)	20 (I)	28 (S)	28 (S)	21 (R)	27 (S)	/
ST47	30 (S)	30 (S)	25 (S)	30 (S)	29 (S)	40 (S)	24 (S)	25 (S)	/
ST48	30 (S)	11 (R)	30 (S)	25 (S)	29 (S)	40 (S)	27 (S)	20 (S)	/
ST49	32 (S)	12 (R)	27 (S)	25 (S)	30 (S)	39 (S)	15 (R)	13 (R)	/
ST50	09 (R)	12 (R)	09 (R)	20 (I)	06 (R)	25 (I)	07 (R)	06 (R)	/
ST51	12 (R)	11 (R)	09 (R)	22 (S)	06 (R)	26 (S)	06 (R)	06 (R)	/
ST52	11 (R)	10 (R)	06 (R)	22 (S)	06 (R)	39 (S)	06 (R)	06 (R)	/
ST53	33 (S)	15 (R)	15 (R)	29 (S)	29 (S)	36 (S)	23 (S)	23 (S)	/
ST54	29 (S)	30 (S)	30 (S)	29 (S)	35 (S)	35 (S)	30 (S)	31 (S)	/
ST55	33 (S)	22 (S)	23(S)	24 (S)	29 (S)	40 (S)	27 (S)	32 (S)	/
ST56	31 (S)	11 (R)	29 (S)	27 (S)	32 (S)	40 (S)	27 (S)	06 (R)	/
ST57	31 (S)	29 (S)	25 (S)	28 (S)	32 (S)	35 (S)	26 (S)	29 (S)	/
ST58	29 (S)	22 (S)	24 (S)	23 (S)	30 (S)	30 (S)	26 (S)	29 (S)	/
ST59	35 (S)	19 (S)	24 (S)	22 (S)	30 (S)	40 (S)	27 (S)	35 (S)	/
ST60	13 (R)	16 (R)	06 (R)	26 (S)	06 (R)	39 (S)	06 (R)	06 (R)	/
ST61	13 (R)	14 (R)	07 (R)	24 (S)	06 (R)	38 (S)	07 (R)	07 (R)	/
ST62	35 (S)	22 (S)	23 (S)	26 (S)	29 (S)	35 (S)	25 (S)	30 (S)	/
ST63	34 (S)	21 (S)	23 (S)	27 (S)	30 (S)	37 (S)	25 (S)	32 (S)	/
ST64	33 (S)	14 (R)	27 (S)	27 (S)	35 (S)	42 (S)	27 (S)	27 (S)	/
ST65	12 (R)	13 (R)	06 (R)	22 (S)	06 (R)	38 (S)	06 (R)	06 (R)	/
ST66	20 (R)	06 (R)	06 (R)	26 (S)	06 (R)	39 (S)	06 (R)	06 (R)	/
ST67	30 (S)	12 (R)	30 (S)	30 (S)	35 (S)	35 (S)	15 (R)	24 (S)	/
ST68	30 (S)	29 (S)	33 (S)	31 (S)	29 (S)	40 (S)	25 (S)	32 (S)	/
ST69	33 (S)	25 (S)	23 (S)	24 (S)	31 (S)	40 (S)	24 (S)	34 (S)	/
ST70	30 (S)	24 (S)	27 (S)	22 (S)	29 (S)	28 (S)	22 (S)	27 (S)	/
ST71	31 (S)	28 (S)	34 (S)	18 (R)	26 (S)	40 (S)	26 (S)	30 (S)	/
ST72	28 (S)	22 (S)	23 (S)	30 (S)	27 (S)	28 (S)	23 (S)	32 (S)	/
ST73	35 (S)	35 (S)	26 (S)	30 (S)	33 (S)	43 (S)	26 (S)	31 (S)	/
ST74	30 (S)	37 (S)	27 (S)	35 (S)	27 (S)	45 (S)	27 (S)	32 (S)	/
ST75	45 (S)	38 (S)	35 (S)	40 (S)	30 (S)	48 (S)	25 (S)	35 (S)	/

S: Sensitive, **R:** resistant, **I:** Intermediate. **Blue box:***S. aureus*.

Table 3C. Average diameters of the zones of inhibition (mm) obtained for the antibiotics tested with regard to the strains of *Staphylococcus* spp. isolates (ST76-ST113).

Strain	Antibiotic (concentration µg)								
	FOX (30)	ERY (15)	GEN (10)	TET (30)	CIP (5)	RIF (5)	TOB (10)	CO-TRI (1.25+23.75)	CFT (30)
ST76	10 (R)	10 (R)	06 (R)	22 (S)	06 (R)	34 (S)	06 (R)	06 (R)	/
ST77	30 (S)	40 (S)	25 (S)	35 (S)	30 (S)	47 (S)	12 (R)	33 (S)	/
ST78	30 (S)	11 (R)	25 (S)	35 (S)	29 (S)	46 (S)	12 (R)	30 (S)	/
ST79	31 (S)	21 (S)	30 (S)	35 (S)	30 (S)	47 (S)	20 (R)	30 (S)	/
ST80	29 (S)	26 (S)	22 (S)	30 (S)	24 (S)	42 (S)	23 (S)	30 (S)	/
ST81	30 (S)	40 (S)	27 (S)	33 (S)	35 (S)	40 (S)	12 (R)	26 (S)	/
ST82	29 (S)	40 (S)	31 (S)	34 (S)	31 (S)	41 (S)	28 (S)	31 (S)	/
ST83	40 (S)	41 (S)	35 (S)	30 (S)	30 (S)	45 (S)	15 (R)	30 (S)	/
ST84	30 (S)	40 (S)	33 (S)	32 (S)	33 (S)	40 (S)	12 (R)	26 (S)	/
ST85	46 (S)	45 (S)	34 (S)	40 (S)	30 (S)	45 (S)	16 (R)	32 (S)	/
ST86	40 (S)	30 (S)	34 (S)	36 (S)	35 (S)	50 (S)	15 (R)	30 (S)	/
ST87	29 (S)	40 (S)	27 (S)	35 (S)	28 (S)	47 (S)	15 (R)	32 (S)	/
ST88	30 (S)	28 (S)	26 (S)	22 (S)	30 (S)	44 (S)	27 (S)	34 (S)	/
ST89	34 (S)	22 (S)	22 (S)	21 (I)	22 (S)	42 (S)	22 (S)	27 (S)	/
ST90	29 (S)	22 (S)	34 (S)	30 (S)	30 (S)	40 (S)	22 (S)	31 (S)	/
ST91	29 (S)	45 (S)	35 (S)	32 (S)	32 (S)	43 (S)	24 (S)	30 (S)	/
ST92	35 (S)	12 (R)	14 (R)	29 (S)	30 (S)	36 (S)	14 (R)	06 (R)	/
ST93	35 (S)	40 (S)	41 (S)	39 (S)	35 (S)	42 (S)	30 (S)	40 (S)	/
ST94	06 (R)	09 (R)	06 (R)	21 (I)	06 (R)	34 (S)	07 (R)	07 (R)	/
ST95	40 (S)	29 (S)	34 (S)	35 (S)	30 (S)	40 (S)	26 (S)	34 (S)	/
ST96	30 (S)	10 (R)	25 (S)	29 (S)	34 (S)	40 (S)	25 (S)	25 (S)	/
ST97	29 (S)	20 (S)	27 (S)	26 (S)	30 (S)	42 (S)	29 (S)	31 (S)	/
ST98	30 (S)	20 (S)	25 (S)	23 (S)	27 (S)	42 (S)	22 (S)	30 (S)	/
ST99	14 (R)	06 (R)	14 (R)	25 (S)	06 (R)	17 (R)	09 (R)	24 (S)	/
ST100	14 (R)	16 (R)	11 (R)	22 (S)	06 (R)	39 (S)	07 (R)	06 (R)	/
ST101	15 (R)	06 (R)	15 (R)	25 (S)	06 (R)	28 (S)	08 (R)	24 (S)	/
ST102	37 (S)	33 (S)	24 (S)	28 (S)	30 (S)	40 (S)	30 (S)	35 (S)	/
ST103	30 (S)	31 (S)	30 (S)	35 (S)	31 (S)	35 (S)	35 (S)	30 (S)	/
ST104	14 (R)	10 (R)	06 (R)	25 (S)	06 (R)	30 (S)	09 (R)	06 (R)	/
ST105	14 (R)	12 (R)	07 (R)	26 (S)	06 (R)	32 (S)	07 (R)	06 (R)	/
ST106	16 (R)	22 (S)	32 (S)	27 (S)	10 (R)	31 (S)	10 (R)	29 (S)	/
ST107	16 (R)	07 (R)	11 (R)	30 (S)	07 (R)	35 (S)	11 (R)	25 (S)	/
ST108	14 (R)	06 (R)	09 (R)	20 (S)	06 (R)	30 (S)	11 (R)	23 (S)	/
ST109	19 (R)	24 (S)	28 (S)	32 (S)	09 (R)	18 (R)	30 (S)	31 (S)	/
ST110	15 (R)	08 (R)	10 (R)	29 (S)	07 (R)	32 (S)	10 (R)	22 (S)	/
ST111	30 (S)	31 (S)	32 (S)	24 (S)	35 (S)	30 (S)	40 (S)	39 (S)	/
ST112	09 (R)	12 (R)	09 (R)	23 (S)	06 (R)	35 (S)	06 (R)	06 (R)	/
ST113	14 (R)	11 (R)	06 (R)	27 (S)	06 (R)	30 (S)	07 (R)	07 (R)	/

S: Sensitive, **R:** resistant, **I:** Intermediate.

Table 3D. Average diameters of the zones of inhibition (mm) obtained for the antibiotics tested with regard to the strains of *Staphylococcus* spp. isolates (ST114-ST143).

Strain	Antibiotic (concentration µg)								
	FOX (30)	ERY (15)	GEN (10)	TET (30)	CIP (5)	RIF (5)	TOB (10)	CO-TRI (1.25+23.75)	CFT (30)
ST114	19 (R)	18 (R)	12 (R)	35 (S)	06 (R)	30 (S)	09 (R)	25 (S)	/
ST115	17 (R)	07 (R)	30 (S)	30 (S)	07 (R)	35 (S)	07 (R)	31 (S)	/
ST116	29 (S)	13 (R)	06 (R)	23 (S)	06 (R)	39 (S)	08 (R)	39 (S)	/
ST117	33 (S)	33 (S)	34 (S)	24 (S)	35 (S)	32 (S)	35 (S)	29 (S)	/
ST118	19 (R)	25 (S)	25 (S)	24 (S)	29 (S)	35 (S)	29 (S)	29 (S)	/
ST119	19 (R)	06 (R)	30 (S)	29 (S)	06 (R)	14 (R)	09 (R)	29 (S)	/
ST120	19 (R)	06 (R)	10 (R)	32 (S)	07 (R)	18 (R)	09 (R)	28 (S)	/
ST121	29 (S)	30 (S)	31 (S)	27 (S)	28 (S)	41 (S)	32 (S)	40 (S)	/
ST122	30 (S)	13 (R)	39 (S)	27 (S)	34 (S)	29 (S)	38 (S)	35 (S)	/
ST123	28 (S)	30 (S)	28 (S)	27 (S)	28 (S)	33 (S)	31 (S)	31 (S)	/
ST124	30 (S)	30 (S)	28 (S)	28 (S)	32 (S)	37 (S)	33 (S)	28 (S)	/
ST125	30 (S)	29 (S)	30 (S)	26 (S)	27 (S)	40 (S)	33 (S)	32 (S)	/
ST126	18 (R)	06 (R)	10 (R)	30 (S)	08 (R)	14 (R)	09 (R)	24 (S)	/
ST127	19 (R)	06 (R)	17 (R)	26 (S)	07 (R)	18 (R)	08 (R)	25 (S)	/
ST128	30 (S)	12 (R)	35 (S)	32 (S)	35 (S)	36 (S)	38 (S)	32 (S)	/
ST129	33 (S)	29 (S)	35 (S)	35 (S)	30 (S)	42 (S)	40 (S)	39 (S)	/
ST130	28 (S)	31 (S)	34 (S)	32 (S)	35 (S)	32 (S)	38 (S)	33 (S)	/
ST131	15 (R)	06 (R)	11 (R)	27 (S)	06 (R)	33 (S)	10 (R)	23 (S)	/
ST132	15 (R)	26 (S)	27 (S)	30 (S)	06 (R)	14 (R)	10 (R)	22 (S)	/
ST133	18 (R)	13 (R)	30 (S)	26 (S)	28 (S)	25 (S)	30 (S)	30 (S)	/
ST134	28 (S)	29 (S)	33 (S)	27 (S)	33 (S)	29 (S)	30 (S)	29 (S)	/
ST135	34 (S)	33 (S)	28 (S)	30 (S)	34 (S)	37 (S)	33 (S)	32 (S)	/
ST136	28 (S)	28 (S)	33 (S)	28 (S)	28 (S)	35 (S)	32 (S)	31 (S)	/
ST137	36 (S)	30 (S)	25 (S)	29 (S)	27 (S)	30 (S)	41 (S)	35 (S)	/
ST138	32 (S)	25 (S)	35 (S)	35 (S)	32 (S)	30 (S)	38 (S)	35 (S)	/
ST139	28 (S)	32 (S)	30 (S)	29 (S)	35 (S)	35 (S)	30 (S)	36 (S)	/
ST140	15 (R)	15 (R)	08 (R)	30 (S)	06 (R)	30 (S)	08 (R)	06 (R)	/
ST141	12 (R)	10 (R)	07 (R)	23 (S)	07 (R)	35 (S)	07 (R)	06 (R)	/
ST142	13 (R)	13 (R)	07 (R)	23 (S)	06 (R)	35 (S)	28 (S)	26 (S)	/
ST143	34 (S)	24 (S)	25 (S)	25 (S)	34 (S)	48 (S)	25 (S)	34 (S)	/

S: Sensitive, **R:** resistant, **I:** Intermediate.

Table 4. Average diameters of the zones of inhibition (mm) obtained for the antibiotics tested with regard to the strains of *Enterococcus faecalis* strains.

Strain	Antibiotic (concentration µg)					
	TET (30)	CIP (5)	ERY (15)	IPM (10)	CO-TRI (1.25+23.75)	RIF (5)
EF1	21 (S)	20 (I)	18 (I)	24 (S)	33 (I)	16 (R)
EF2	23 (S)	20 (I)	18 (I)	28 (S)	36 (I)	18 (I)
EF3	22 (S)	20 (I)	16 (I)	25 (S)	33 (I)	20 (S)
EF4	24 (S)	19 (I)	17 (I)	30 (S)	34 (I)	16 (R)
EF5	08 (R)	20 (I)	20 (I)	29 (S)	39 (I)	21 (S)
EF6	08 (R)	08 (R)	06 (R)	34 (S)	36 (I)	25 (S)
EF7	09 (R)	08 (R)	06 (R)	29 (S)	29 (I)	24 (S)
EF8	07 (R)	06 (R)	06 (R)	30 (S)	21 (I)	18 (I)
EF9	08 (R)	08 (R)	06 (R)	29 (S)	21 (I)	19 (I)
EF10	08 (R)	09 (R)	06 (R)	24 (S)	24 (I)	20 (S)
EF11	08 (R)	08 (R)	06 (R)	30 (S)	38 (I)	22 (S)
EF12	09 (R)	08 (R)	08 (R)	24 (S)	29 (I)	18 (I)
EF13	09 (R)	10 (R)	06 (R)	27 (S)	26 (I)	21 (S)
EF14	08 (R)	09 (R)	06 (R)	27 (S)	27 (I)	18 (I)
EF15	06 (R)	09 (R)	06 (R)	24 (S)	23 (I)	17 (I)
EF16	07 (R)	06 (R)	06 (R)	24 (S)	24 (I)	20 (S)
EF17	07 (R)	11 (R)	06 (R)	25 (S)	25 (I)	20 (S)
EF18	07 (R)	08 (R)	06 (R)	25 (S)	25 (I)	21 (S)
EF19	08 (R)	10 (R)	06 (R)	24 (S)	23 (I)	21 (S)
EF20	08 (R)	10 (R)	06 (R)	24 (S)	25 (I)	21 (S)
EF21	07 (R)	10 (R)	06 (R)	25 (S)	25 (I)	22 (S)
EF22	08 (R)	08 (R)	06 (R)	25 (S)	25 (I)	22 (S)
EF23	07 (R)	14 (R)	06 (R)	26 (S)	26 (I)	24 (S)
EF24	07 (R)	07 (R)	06 (R)	27 (S)	32 (I)	20 (S)
EF25	07 (R)	09 (R)	06 (R)	25 (S)	27 (I)	15 (R)
EF26	06 (R)	08 (R)	06 (R)	22 (S)	22 (I)	17 (I)
EF27	07 (R)	09 (R)	06 (R)	23 (S)	18 (R)	12 (R)
EF28	07 (R)	08 (R)	06 (R)	19 (I)	21 (I)	20 (S)
EF29	07 (R)	06 (R)	06 (R)	24 (S)	22 (I)	18 (I)
EF30	07 (R)	10 (R)	06 (R)	26 (S)	27 (I)	20 (S)
EF31	08 (R)	11 (R)	06 (R)	25 (S)	23 (I)	16 (R)
EF32	08 (R)	21 (S)	25 (S)	26 (S)	27 (I)	18 (I)
EF33	10 (R)	09 (R)	06 (R)	23 (S)	30 (I)	18 (I)

S:Sensitive, **R:** resistant, **I:** Intermediate.

Table 5. MIC (µg/mL) obtained for vancomycin tested regard *Enterococcus faecalis* strains using E-test.

Strains	E-test MIC (µg/mL)	Strains	E-test MIC (µg/mL)
EF1	1.5	**EF18**	1.5
EF2	0.75	**EF19**	1
EF3	0.5	**EF20**	1
EF4	1	**EF21**	1
EF5	0.75	**EF22**	0.75
EF6	1	**EF23**	1
EF7	0.75	**EF24**	0.75
EF8	1	**EF25**	1
EF9	1	**EF26**	0.75
EF10	0.75	**EF27**	1
EF11	1	**EF28**	1.5
EF12	0.75	**EF29**	1
EF13	1.5	**EF30**	1.5
EF14	0.75	**EF31**	1
EF15	0.75	**EF32**	1
EF16	1	**EF33**	1.5
EF17	1		

Appendix IX

Table 1A. Bacterial cell surface hydrophobicity and auto-aggregation of *Enterobacteriaceae* strains (EC1-EC29) isolated from medical devices. The values are the means of three independent experiments.

Identity	Hydrophobicity (%)		Auto-aggregation (%)
Escherichia coli EC1	32.03±0.012	Moderately hydrophobic	12.07±0.028
Escherichia coli EC2	14.39±0.021	Hydrophilic	18.10±0.027
Escherichia coli EC3	18.63±0.013	Hydrophilic	15.69±0.017
Escherichia coli EC4	20.10±0.008	Moderately hydrophobic	19.02±0.012
Escherichia coli EC5	15.03±0.029	Hydrophilic	18.60±0.017
Escherichia coli EC6	29.28±0.024	Moderately hydrophobic	9.46±0.018
Escherichia coli EC7	3.08±0.016	Hydrophilic	18.19±0.041
Escherichia coli EC8	5.43±0.017	Hydrophilic	4.15±0.008
Escherichia coli EC9	12.20±0.026	Hydrophilic	78.21±0.031
Escherichia coli EC10	9.70±0.025	Hydrophilic	16.40±0.021
Escherichia coli EC11	3.37±0.008	Hydrophilic	12.46±0.033
Escherichia coli EC12	12.31±0.019	Hydrophilic	7.46±0.021
Escherichia coli EC13	10.42±0.030	Hydrophilic	18.15±0.019
Escherichia coli EC14	6.02±0.012	Hydrophilic	22.87±0.015
Escherichia coli EC15	2.67±0.012	Hydrophilic	21.18±0.020
Escherichia coli EC16	3.43±0.005	Hydrophilic	12.69±0.010
Escherichia coli EC17	17.78±0.008	Hydrophilic	12.44±0.011
Escherichia coli EC18	4.59±0.003	Hydrophilic	13.60±0.019
Escherichia coli EC19	10.61±0.015	Hydrophilic	8.78±0.020
Escherichia coli EC20	29.38±0.004	Moderately hydrophobic	8.59±0.014
Escherichia coli EC21	16.24±0.012	Hydrophilic	9.21±0.013
Escherichia coli EC22	40.01±0.015	Moderately hydrophobic	7.59±0.033
Escherichia coli EC23	35.96±0.014	Moderately hydrophobic	4.67±0.008
Escherichia coli EC24	31.01±0.008	Moderately hydrophobic	7.96±0.006
Escherichia coli EC25	44.17±0.017	Moderately hydrophobic	23.23±0.004
Escherichia coli EC26	48.91±0.028	Moderately hydrophobic	7.19±0.017
Escherichia coli EC27	31.43±0.020	Moderately hydrophobic	6.24±0.018
Escherichia coli EC28	29.25±0.012	Moderately hydrophobic	18.50±0.015
Escherichia coli EC29	20.73±0.010	Moderately hydrophobic	8.56±0.015

Table 1B. Bacterial cell surface hydrophobicity and auto-aggregation of *Enterobacteriaceae* strains (EC30-KP6) isolated from medical devices. The values are the means of three independent experiments.

Identity	Hydrophobicity (%)		Auto-aggregation (%)
Escherichia coli EC30	11.57±0.004	Hydrophilic	7.00±0.021
Escherichia coli EC31	8.69±0.007	Hydrophilic	10.60±0.015
Klebsiella pneumoniae KP1	9.34±0.015	Hydrophilic	14.60±0.020
Klebsiella pneumoniae KP2	4.50±0.011	Hydrophilic	11.78±0.012
Klebsiella pneumoniae KP3	4.94±0.006	Hydrophilic	27.48±0.018
Klebsiella pneumoniae KP4	30.82±0.005	Moderately hydrophobic	15.86±0.013
Klebsiella pneumoniae KP5	40.51±0.010	Moderately hydrophobic	9.68±0.017
Klebsiella pneumoniae KP6	17.75±0.003	Hydrophilic	11.30±0.034

Table 2. Bacterial cell surface hydrophobicity and auto-aggregation of *Acinetobacter* spp. strains isolated from medical devices. The values are the means of three independent experiments.

Identity	Hydrophobicity (%)		Auto-agregation (%)
Acinetobacter baumannii AB1	10.50±0.019	Hydrophilic	17.89±0.021
Acinetobacter baumannii AB2	6.27±0.003	Hydrophilic	20.78±0.026
Acinetobacter baumanniiAB3	14.77±0.021	Hydrophilic	1.57±0.007
Acinetobacter lwoffii AL	59.80±0.014	Hydrophobic	3.46±0.006
Acinetobacter baumannii AB4	5.50±0.008	Hydrophilic	15.08±0.013
Acinetobacter baumannii AB5	0.92±0.004	Hydrophilic	5.77±0.026

Table 3A. Bacterial cell surface hydrophobicity and auto-aggregation of *Staphylococcus* spp. strains (ST1-ST30) isolated from medical devices. The values are the means of three independent experiments.

Identity	Hydrophobicity (%)		Auto-aggregation (%)
***Staphylococcus aureus* ST1**	34.81±0.021	Moderately hydrophobic	15.18±0.027
***Staphylococcus aureus* ST2**	81.04±0.024	Hydrophobic	6.52±0.021
***Staphylococcus aureus* ST3**	62.64±0.028	Hydrophobic	7.90±0.010
***Staphylococcus cohnii* ST4**	31.37±0.032	Moderately hydrophobic	24.16±0.029
***Staphylococcus* spp.ST5**	45.03±0.028	Moderately hydrophobic	31.19±0.013
***Staphylococcus* spp. ST6**	67.05±0.015	Hydrophobic	21.12±0.036
***Staphylococcus* spp.ST7**	82.53±0.029	Hydrophobic	36.61±0.032
***Staphylococcus aureus* ST8**	27.46±0.025	Moderately hydrophobic	20.61±0.011
***Staphylococcus aureus* ST9**	27.17±0.012	Moderately hydrophobic	13.66±0.009
***Staphylococcus aureus* ST10**	80.48±0.025	Hydrophobic	15.54±0.021
***Staphylococcus aureus* ST11**	78.06±0.010	Hydrophobic	10.41±0.019
***Staphylococcus aureus* ST12**	41.75±0.033	Moderately hydrophobic	12.74±0.027
***Staphylococcus aureus* ST13**	64.56±0.032	Hydrophobic	29.20±0.004
***Staphylococcus aureus* ST14**	43.17±0.033	Moderately hydrophobic	10.43±0.025
***Staphylococcus aureus* ST15**	55.03±0.014	Hydrophobic	6.70±0.009
***Staphylococcus aureus* ST16**	59.09±0.012	Hydrophobic	12.99±0.014
***Staphylococcus aureus* ST17**	78.82±0.028	Hydrophobic	12.74±0.021
***Staphylococcus aureus* ST18**	48.62±0.020	Moderately hydrophobic	5.11±0.019
***Staphylococcus aureus* ST19**	56.28±0.014	Hydrophobic	9.45±0.014
***Staphylococcus aureus* ST20**	80.51±0.019	Hydrophobic	10.26±0.025
***Staphylococcus aureus* ST21**	55.49±0.012	Hydrophobic	12.32±0.013
***Staphylococcus* spp.ST22**	68.93±0.014	Hydrophobic	19.73±0.013
***Staphylococcus* spp.ST23**	83.94±0.020	Hydrophobic	16.96±0.009
***Staphylococcus* spp.ST24**	67.94±0.016	Hydrophobic	10.52±0.023
***Staphylococcus* spp.ST25**	56.11±0.013	Hydrophobic	14.47±0.035
***Staphylococcus* spp.ST26**	49.56±0.011	Moderately hydrophobic	2.53±0.009
***Staphylococcus aureus* ST27**	21.01±0.008	Moderately hydrophobic	10.76±0.011
***Staphylococcus* spp.ST28**	11.74±0.002	Hydrophilic	10.89±0.016
***Staphylococcus* spp.ST29**	69.16±0.009	Hydrophobic	8.71±0.003
***Staphylococcus* spp.ST30**	30.84±0.008	Moderately hydrophobic	14.51±0.021

Table 3B. Bacterial cell surface hydrophobicity and auto-aggregation of *Staphylococcus* spp. strains (ST31-ST60) isolated from medical devices. The values are the means of three independent experiments.

Identity	Hydrophobicity (%)		Auto-aggregation (%)
***Staphylococcus haemolyticus* ST31**	31.77±0.010	Moderately hydrophobic	10.92±0.017
***Staphylococcus haemolyticus* ST32**	58.20±0.009	Hydrophobic	7.18±0.015
***Staphylococcus haemolyticus* ST33**	81.36±0.010	Hydrophobic	20.40±0.020
***Staphylococcus aureus* ST34**	24.92±0.017	Moderately hydrophobic	12.67±0.002
***Staphylococcus aureus* ST35**	13.46±0.011	Hydrophilic	10.36±0.017
***Staphylococcus* spp. ST36**	74.16±0.023	Hydrophobic	14.73±0.021
***Staphylococcus* spp. ST37**	69.95±0.010	Hydrophobic	19.46±0.014
***Staphylococcus* spp. ST38**	1.43±0.004	Hydrophilic	15.94±0.024
***Staphylococcus* spp. ST39**	48.50±0.017	Moderately hydrophobic	16.10±0.021
***Staphylococcus aureus* ST40**	45.86±0.020	Moderately hydrophobic	13.07±0.015
***Staphylococcus* spp. ST41**	58.11±0.021	Hydrophobic	10.66±0.019
***Staphylococcus aureus* ST42**	63.74±0.028	Hydrophobic	14.01±0.016
***Staphylococcus aureus* ST43**	70.17±0.027	Hydrophobic	13.73±0.014
***Staphylococcus aureus* ST44**	71.25±0.016	Hydrophobic	19.18±0.024
***Staphylococcus* spp. ST45**	50.67±0.011	Hydrophobic	19.02±0.014
***Staphylococcus* spp. ST46**	42.64±0.033	Moderately hydrophobic	7.53±0.014
***Staphylococcus* spp. ST47**	58.58±0.021	Hydrophobic	20.41±0.017
***Staphylococcus* spp. ST48**	7.34±0.018	Hydrophilic	30.47±0.023
***Staphylococcus* spp. ST49**	6.16±0.019	Hydrophilic	40.77±0.022
***Staphylococcus* spp. ST50**	4.06±0.004	Hydrophilic	19.14±0.019
***Staphylococcus* spp. ST51**	35.58±0.015	Moderately hydrophobic	22.26±0.008
***Staphylococcus* spp. ST52**	70.13±0.028	Hydrophobic	21.90±0.028
***Staphylococcus* spp. ST53**	37.32±0.026	Moderately hydrophobic	32.87±0.034
***Staphylococcus* spp. ST54**	73.47±0.027	Hydrophobic	40.13±0.016
***Staphylococcus* spp. ST55**	57.54±0.020	Hydrophobic	19.59±0.006
***Staphylococcus* spp. ST56**	15.74±0.011	Hydrophilic	13.36±0.032
***Staphylococcus* spp. ST57**	47.05±0.035	Moderately hydrophobic	20.77±0.031
***Staphylococcus* spp. ST58**	4.41±0.013	Hydrophilic	11.81±0.018
***Staphylococcus* spp. ST59**	55.39±0.026	Hydrophobic	19.28±0.009
***Staphylococcus* spp. ST60**	70.86±0.024	Hydrophobic	22.27±0.018

Table 3C. Bacterial cell surface hydrophobicity and auto-aggregation of *Staphylococcus* spp. strains (ST61-ST90) isolated from medical devices. The values are the means of three independent experiments.

Identity	Hydrophobicity (%)		Auto-aggregation (%)
***Staphylococcus* spp. ST61**	40.27±0.014	Moderately hydrophobic	23.18±0.027
***Staphylococcus* spp. ST62**	18.28±0.028	Hydrophilic	17.12±0.013
***Staphylococcus* spp. ST63**	34.28±0.002	Moderately hydrophobic	30.80±0.022
***Staphylococcus* spp. ST64**	80.57±0.033	Hydrophobic	27.53±0.027
***Staphylococcus* spp. ST65**	64.88±0.016	Hydrophobic	28.02±0.019
***Staphylococcus* spp. ST66**	10.89±0.027	Hydrophilic	11.90±0.038
***Staphylococcus* spp. ST67**	2.99±0.016	Hydrophilic	14.08±0.036
***Staphylococcus* spp. ST68**	61.07±0.022	Hydrophobic	6.25±0.025
***Staphylococcus* spp. ST69**	66.73±0.016	Hydrophobic	23.31±0.037
***Staphylococcus* spp. ST70**	65.19±0.033	Hydrophobic	20.44±0.039
***Staphylococcus* spp. ST71**	69.86±0.033	Hydrophobic	12.75±0.029
***Staphylococcus* spp. ST72**	36.60±0.024	Moderately hydrophobic	45.11±0.015
***Staphylococcus* spp. ST73**	25.73±0.027	Moderately hydrophobic	19.39±0.038
***Staphylococcus* spp. ST74**	35.38±0.036	Moderately hydrophobic	8.39±0.029
***Staphylococcus* spp. ST75**	47.72±0.033	Moderately hydrophobic	14.26±0.036
***Staphylococcus* spp. ST76**	82.16±0.031	Hydrophobic	19.48±0.007
***Staphylococcus* spp. ST77**	62.95±0.010	Hydrophobic	8.92±0.020
***Staphylococcus* spp. ST78**	25.58±0.017	Moderately hydrophobic	10.59±0.018
***Staphylococcus* spp. ST79**	21.03±0.031	Moderately hydrophobic	4.02±0.021
***Staphylococcus* spp. ST80**	72.79±0.007	Hydrophobic	20.60±0.023
***Staphylococcus* spp. ST81**	28.08±0.017	Moderately hydrophobic	27.59±0.012
***Staphylococcus* spp. ST82**	12.23±0.018	Hydrophilic	23.79±0.034
***Staphylococcus* spp. ST83**	8.67±0.028	Hydrophilic	17.08±0.035
***Staphylococcus* spp. ST84**	31.20±0.023	Moderately hydrophobic	48.34±0.033
***Staphylococcus* spp. ST85**	16.15±0.026	Hydrophilic	16.63±0.018
***Staphylococcus* spp. ST86**	70.44±0.024	Hydrophobic	43.09±0.019
***Staphylococcus* spp. ST87**	3.41±0.010	Hydrophilic	8.26±0.017
***Staphylococcus* spp. ST88**	72.28±0.032	Hydrophobic	12.08±0.024
***Staphylococcus* spp. ST89**	33.77±0.005	Moderately hydrophobic	16.77±0.017
***Staphylococcus* spp. ST90**	18.57±0.027	Hydrophilic	14.36±0.024

Table 3D. Bacterial cell surface hydrophobicity and auto-aggregation of *Staphylococcus* spp. strains (ST91-ST120) isolated from medical devices. The values are the means of three independent experiments.

Identity	Hydrophobicity (%)		Auto-aggregation (%)
***Staphylococcus* spp. ST91**	15.54±0.031	Hydrophilic	17.47±0.028
***Staphylococcus* spp. ST92**	54.79±0.022	Hydrophobic	14.06±0.035
***Staphylococcus* spp. ST93**	53.54±0.031	Hydrophobic	25.26±0.019
***Staphylococcus* spp. ST94**	59.30±0.027	Hydrophobic	17.37±0.028
***Staphylococcus* spp. ST95**	27.11±0.019	Moderately hydrophobic	26.80±0.015
***Staphylococcus* spp. ST96**	49.26±0.018	Moderately hydrophobic	12.70±0.021
***Staphylococcus* spp. ST97**	76.34±0.024	Hydrophobic	12.15±0.014
***Staphylococcus* spp. ST98**	44.75±0.012	Moderately hydrophobic	13.61±0.014
***Staphylococcus* spp. ST99**	24.33±0.025	Moderately hydrophobic	2.94±0.004
***Staphylococcus* spp. ST100**	69.14±0.008	Hydrophobic	20.84±0.029
***Staphylococcus* spp. ST101**	73.33±0.016	Hydrophobic	12.54±0.024
***Staphylococcus* spp. ST102**	43.48±0.032	Moderately hydrophobic	15.30±0.024
***Staphylococcus* spp. ST103**	33.40±0.021	Moderately hydrophobic	8.22±0.021
***Staphylococcus* spp. ST104**	46.92±0.025	Moderately hydrophobic	14.38±0.033
***Staphylococcus* spp. ST105**	53.12±0.030	Hydrophobic	13.26±0.028
***Staphylococcus* spp. ST106**	47.25±0.011	Moderately hydrophobic	22.55±0.023
***Staphylococcus* spp. ST107**	24.82±0.016	Moderately hydrophobic	19.38±0.023
***Staphylococcus* spp. ST108**	4.54±0.012	Hydrophilic	25.04±0.031
***Staphylococcus* spp. ST109**	33.97±0.028	Moderately hydrophobic	11.28±0.021
***Staphylococcus* spp. ST110**	25.22±0.009	Moderately hydrophobic	7.09±0.027
***Staphylococcus* spp. ST111**	53.81±0.026	Hydrophobic	14.12±0.022
***Staphylococcus* spp. ST112**	65.83±0.023	Hydrophobic	28.01±0.032
***Staphylococcus* spp. ST113**	51.45±0.031	Hydrophobic	18.91±0.027
***Staphylococcus* spp. ST114**	20.19±0.019	Moderately hydrophobic	14.88±0.031
***Staphylococcus* spp. ST115**	5.94±0.022	Hydrophilic	14.45±0.009
***Staphylococcus* spp. ST116**	92.99±0.025	Hydrophobic	9.35±0.009
***Staphylococcus* spp. ST117**	53.52±0.026	Hydrophobic	13.89±0.032
***Staphylococcus* spp. ST118**	66.97±0.023	Hydrophobic	16.52±0.010
***Staphylococcus* spp. ST119**	5.24±0.021	Hydrophilic	6.51±0.021
***Staphylococcus* spp. ST120**	49.01±0.005	Moderately hydrophobic	10.01±0.016

Table 3E. Bacterial cell surface hydrophobicity and auto-aggregation of *Staphylococcus* spp. strains (ST121-ST143) isolated from medical devices. The values are the means of three independent experiments.

Identity	Hydrophobicity (%)		Auto-aggregation (%)
***Staphylococcus* spp. ST121**	68.78±0.026	Hydrophobic	14.37±0.003
***Staphylococcus* spp. ST122**	43.33±0.009	Moderately hydrophobic	13.99±0.025
***Staphylococcus* spp. ST123**	36.19±0.014	Moderately hydrophobic	15.69±0.012
***Staphylococcus* spp. ST124**	22.01±0.017	Moderately hydrophobic	15.39±0.021
***Staphylococcus* spp. ST125**	33.30±0.017	Moderately hydrophobic	17.21±0.022
***Staphylococcus* spp. ST126**	3.24±0.018	Hydrophilic	8.84±0.013
***Staphylococcus* spp. ST127**	48.24±0.011	Moderately hydrophobic	17.44±0.008
***Staphylococcus* spp. ST128**	24.88±0.016	Moderately hydrophobic	23.33±0.006
***Staphylococcus* spp. ST129**	25.83±0.013	Moderately hydrophobic	16.33±0.016
***Staphylococcus* spp. ST130**	31.67±0.013	Moderately hydrophobic	18.04±0.005
***Staphylococcus* spp. ST131**	2.15±0.007	Hydrophilic	12.10±0.012
***Staphylococcus* spp. ST132**	2.80±0.012	Hydrophilic	4.73±0.015
***Staphylococcus* spp. ST133**	15.29±0.007	Hydrophilic	16.11±0.034
***Staphylococcus* spp. ST134**	39.73±0.024	Moderately hydrophobic	11.69±0.020
***Staphylococcus* spp. ST135**	69.81±0.021	Hydrophobic	22.12±0.026
***Staphylococcus* spp. ST136**	56.13±0.022	Hydrophobic	13.78±0.025
***Staphylococcus* spp. ST137**	67.75±0.020	Hydrophobic	21.42±0.016
***Staphylococcus* spp. ST138**	45.23±0.014	Moderately hydrophobic	15.68±0.011
***Staphylococcus* spp. ST139**	36.00±0.023	Moderately hydrophobic	21.54±0.019
***Staphylococcus* spp. ST140**	42.23±0.015	Moderately hydrophobic	15.00±0.025
***Staphylococcus* spp. ST141**	53.76±0.021	Hydrophobic	19.47±0.016
***Staphylococcus* spp. ST142**	14.44±0.023	Moderately hydrophobic	14.00±0.017
***Staphylococcus* spp. ST143**	37.15±0.015	Moderately hydrophobic	15.18±0.027

Table 4A. Bacterial cell surface hydrophobicity and auto-aggregation of *Enterococcus faecalis* strains (EF1-EF30) isolated from medical devices. The values are the means of three independent experiments.

Identity	Hydrophobicity (%)		Auto-aggregation (%)
***Enteroococcus faecalis* EF1**	10.17±0.028	Hydrophilic	15.05±0.009
***Enteroococcus faecalis* EF2**	4.10±0.013	Hydrophilic	11.78±0.038
***Enteroococcus faecalis* EF3**	45.84±0.029	Moderately hydrophobic	22.65±0.008
***Enteroococcus faecalis* EF4**	32.51±0.019	Moderately hydrophobic	23.09±0.014
***Enteroococcus faecalis* EF5**	23.07±0.019	Moderately hydrophobic	22.93±0.016
***Enteroococcus faecalis* EF6**	40.94±0.014	Moderately hydrophobic	25.81±0.013
***Enteroococcus faecalis* EF7**	43.65±0.037	Moderately hydrophobic	17.08±0.024
***Enteroococcus faecalis* EF8**	5.33±0.027	Hydrophilic	20.70±0.008
***Enteroococcus faecalis* EF9**	55.64±0.034	Hydrophobic	29.45±0.013
***Enteroococcus faecalis* EF10**	46.62±0.028	Moderately hydrophobic	14.90±0.018
***Enteroococcus faecalis* EF11**	46.61±0.025	Moderately hydrophobic	14.92±0.029
***Enteroococcus faecalis* EF12**	36.78±0.023	Moderately hydrophobic	7.66±0.008
***Enteroococcus faecalis* EF13**	18.22±0.039	Hydrophilic	19.58±0.025
***Enteroococcus faecalis* EF14**	63.86±0.027	Hydrophobic	20.54±0.029
***Enteroococcus faecalis* EF15**	49.95±0.025	Moderately hydrophobic	39.26±0.007
***Enteroococcus faecalis* EF16**	32.57±0.029	Moderately hydrophobic	21.76±0.014
***Enteroococcus faecalis* EF17**	63.47±0.027	Hydrophobic	17.74±0.013
***Enteroococcus faecalis* EF18**	26.61±0.024	Moderately hydrophobic	9.93±0.013
***Enteroococcus faecalis* EF19**	6.32±0.024	Hydrophilic	12.42±0.018
***Enteroococcus faecalis* EF20**	9.45±0.013	Hydrophilic	11.77±0.009
***Enteroococcus faecalis* EF21**	19.50±0.028	Hydrophilic	6.68±0.007
***Enteroococcus faecalis* EF22**	76.49±0.027	Hydrophobic	14.91±0.014
***Enteroococcus faecalis* EF23**	21.35±0.023	Moderately hydrophobic	16.02±0.023
***Enteroococcus faecalis* EF24**	12.51±0.023	Hydrophilic	13.44±0.030
***Enteroococcus faecalis* EF25**	21.57±0.029	Moderately hydrophobic	16.31±0.017
***Enteroococcus faecalis* EF26**	40.42±0.024	Moderately hydrophobic	19.31±0.012
***Enteroococcus faecalis* EF27**	33.75±0.036	Moderately hydrophobic	10.68±0.009
***Enteroococcus faecalis* EF28**	28.72±0.027	Moderately hydrophobic	11.25±0.016
***Enteroococcus faecalis* EF29**	54.57±0.038	Hydrophobic	19.83±0.007
***Enteroococcus faecalis* EF30**	16.99±0.008	Hydrophilic	11.20±0.008

Table 4B. Bacterial cell surface hydrophobicity and auto-aggregation of *Enterococcus faecalis* strains (EF31-EF33) isolated from medical devices. The values are the means of three independent experiments.

Identity	Hydrophobicity (%)		Auto-aggregation (%)
***Enteroococcus faecalis* EF31**	22.61±0.022	Moderately hydrophobic	11.82±0.013
***Enteroococcus faecalis* EF32**	42.74±0.024	Moderately hydrophobic	13.51±0.018
***Enteroococcus faecalis* EF33**	6.49±0.010	Hydrophilic	15.32±0.003

Appendix X

Table 1. Co-aggregation of Gram- negative and Gram- positive bacterial strains isolated from medical devices. The values are the means of three independent experiments. **ST:** *Staphylococcus* spp., **EF:** *En. faecalis*.

Strain X	Strain Y	Co-aggregation (%)
E. coli EC1	ST62	11.37±0.019
	ST70	23.74±0.040
	ST40	12.96±0.034
	EF17	30.67±0.019
EC2	ST66	2.05±0.013
	ST46	9.29±0.011
	ST58	30.22±0.034
	ST37	56.62±0.037
EC3	ST67	1.51±0.008
	ST98	15.37±0.047
	ST47	54.50±0.038
	ST44	20.55±0.035
EC4	ST68	4.41±0.021
	ST102	36.33±0.022
	ST116	10.54±0.015
	EF3	48.18±0.036
EC5	ST69	7.36±0.027
	ST103	30.83±0.032
	ST134	33.49±0.025
	EF4	29.34±0.025
EC6	ST48	6.87±0.012
	ST72	7.67±0.021
	ST107	3.71±0.009
	EF19	17.18±0.035
EC7	ST49	12.64±0.037
	ST108	12.71±0.033
	ST111	15.03±0.035
	ST7	13.73±0.020
	EF27	11.63±0.035
EC8	ST52	42.01±0.022
	ST96	20.41±0.019
	ST4	41.60±0.033
	ST45	36.33±0.021
	EF20	15.49±0.028
EC9	ST54	36.30±0.021
	ST74	25.19±0.021
	ST5	4.70±0.019
	EF11	26.66±0.037
EC10	ST73	17.02±0.026
	ST125	8.84±0.028
	ST6	31.93±0.032
	EF12	29.94±0.018
EC11	ST75	20.73±0.024
	ST126	1.73±0.013
	ST8	9.93±0.014
	EF28	29.06±0.039

Table 2. Co-aggregation of Gram- negative and Gram- positive bacterial strains isolated from medical devices. The values are the means of three independent experiments. **ST:** *Staphylococcus* spp., **EF:** *En. faecalis*.

Strain X	Strain Y	Co-aggregation (%)
E. coli EC12	ST76	3.29±0.011
	ST127	10.32±0.024
	ST9	7.57±0.008
	EF29	11.25±0.034
EC13	ST77	15.10±0.029
	ST128	2.37±0.008
	ST133	6.63±0.028
	ST10	7.83±0.024
	EF21	15.81±0.019
EC14	ST78	4.88±0.020
	ST129	16.36±0.017
	ST11	10.51±0.021
	EF26	36.03±0.035
EC15	ST79	12.69±0.026
	ST130	2.56±0.007
	ST12	26.51±0.037
	EF33	19.18±0.031
EC16	ST131	16.46±0.026
	ST139	36.56±0.043
	ST13	26.46±0.041
	EF32	14.10±0.035
EC17	ST83	4.73±0.031
	ST137	28.30±0.032
	ST17	38.97±0.039
	EF15	40.79±0.031
EC18	ST84	2.75±0.013
	ST115	2.73±0.012
	ST138	5.29±0.019
	ST18	16.71±0.039
EC19	ST85	2.46±0.011
	ST140	53.71±0.036
	ST19	42.28±0.041
	EF14	23.42±0.021
EC20	ST86	6.06±0.025
	ST141	6.47±0.021
	ST20	37.41±0.033
	EF13	21.12±0.023
EC21	ST87	29.83±0.035
	ST142	8.29±0.026
	ST21	5.47±0.020
	EF30	17.70±0.038
EC22	ST89	11.29±0.024
	ST143	18.08±0.021
	ST22	16.61±0.025
	ST36	12.77±0.032

Table 3. Co-aggregation of Gram- negative and Gram- positive bacterial strains isolated from medical devices. The values are the means of three independent experiments. **ST:** *Staphylococcus* spp., **EF:** *En. faecalis*.

Strain X	Strain Y	Co-aggregation (%)
E. coli EC23	ST50	1.73±0.016
	ST92	8.27±0.013
	ST25	28.33±0.022
	ST97	56.19±0.023
EC24	ST51	23.94±0.015
	ST93	8.14±0.021
	ST26	39.95±0.030
	ST118	6.58±0.027
EC25	ST59	69.43±0.033
	ST94	19.47±0.037
	ST119	3.52±0.023
	ST27	11.33±0.034
EC26	ST53	6.16±0.017
	ST95	52.50±0.026
	ST28	13.32±0.032
	EF6	27.07±0.004
EC27	ST113	3.34±0.017
	ST120	4.64±0.011
	ST30	26.17±0.036
	EF7	1.26±0.009
EC28	ST55	2.27±0.014
	ST114	13.20±0.033
	ST121	2.89±0.013
	ST29	70.48±0.024
EC29	ST56	12.33±0.039
	ST3	46.80±0.028
	ST32	25.39±0.029
	EF10	16.95±0.026
EC30	ST105	27.15±0.022
	ST124	4.62±0.020
	ST2	23.47±0.043
	EF8	16.06±0.029
EC31	ST81	5.79±0.016
	ST135	2.51±0.007
	ST15	38.03±0.037
	EF22	15.98±0.022
K. pneumoniae KP1	ST100	18.92±0.022
	ST112	23.86±0.021
	ST43	7.74±0.021
	EF24	43.11±0.021
KP2	ST61	23.63±0.022
	ST64	30.83±0.036
	ST101	17.20±0.034
	EF25	34.21±0.036

Table 4. Co-aggregation of Gram- negative and Gram- positive bacterial strains isolated from medical devices. The values are the means of three independent experiments. **ST:** *Staphylococcus* spp., **EF:** *En. faecalis.*

Strain X	Strain Y	Co-aggregation (%)
K. pneumoniae KP2	ST61	23.63±0.022
	ST64	30.83±0.036
	ST101	17.20±0.034
	EF25	34.21±0.036
KP3	ST80	38.29±0.028
	ST132	5.30±0.013
	ST14	42.04±0.035
	EF31	15.57±0.045
KP4	ST90	4.26±0.020
	ST110	12.26±0.029
	ST23	3.64±0.025
	EF1	30.80±0.017
KP5	ST91	12.71±0.020
	ST24	8.01±0.014
	ST38	2.06±0.007
	EF2	35.14±0.030
KP6	ST82	31.18±0.015
	ST136	10.89±0.031
	ST16	2.24±0.007
	ST35	4.46±0.021
A.baumannii AB1	ST88	7.92±0.003
	ST123	19.55±0.031
	ST40	58.20±0.015
	EF16	46.98±0.034
AB2	ST63	18.52±0.038
	ST71	2.93±0.007
	ST41	36.18±0.036
	EF18	21.74±0.029
AB3	ST99	16.82±0.030
	ST109	13.83±0.038
	ST42	6.95±0.021
	EF23	18.00±0.025
AB4	ST65	45.86±0.051
	ST104	11.01±0.037
	ST106	5.86±0.025
	ST1	17.75±0.017
	EF5	32.16±0.039
AB5	ST57	7.72±0.030
	ST117	20.34±0.025
	ST33	13.57±0.029
	EF9	25.97±0.037
A. lwoffii AL	ST60	12.90±0.024
	ST122	22.04±0.024
	ST31	2.01±0.009
	ST34	3.84±0.012

Glossary

Glossary

Abscess: A local accumulation of pus anywhere in the body **(Shiel *et al*., 2008).**

Appendicitis: Inflammation of the appendix usually associated with infection of the appendix. Appendicitis often causes fever, loss of appetite, and pain **(Shiel *et al*., 2008).**

Asthma: A common lung disorder in which inflammation causes the bronchi to swell and narrow the airways, creating breathing difficulties that may range from mild to life-threatening. Symptoms include shortness of breath, cough, wheezing, and chest tightness. The diagnosis of asthma is based on evidence of wheezing and is confirmed with breathing tests **(Shiel *et al*., 2008).**

Atrium: An entry chamber. On both sides of the heart, the atrium is the chamber that leads to the ventricle **(Shiel *et al*., 2008).**

Bacteremia: The presence of live bacteria in the bloodstream **(Shiel *et al*., 2008).**

Bacteriuria: A condition in which bacteria are present in the urine **(Bateman *et al*., 2007).**

Chemotherapy: Pertaining to treatment with drugs to kill cancer cells. Most anticancer drugs are injected into a vein, but some are given by mouth. Chemotherapy is usually systemic treatment, meaning that the drugs flow through the bloodstream to nearly every part of the body **(Shiel *et al*., 2008).**

Colon cancer: A malignant tumor arising from the inner wall of the large intestine (the colon) **(Shiel *et al*., 2008).**

Crohn's disease: A chronic inflammatory bowel disease that primarily involves the small and/or large intestine. Crohn's disease can be a chronic, recurrent condition, or it can cause minimal symptoms. In mild forms, Crohn's disease causes scattered, shallow, ulcers in the inner surface of the bowel. In more serious cases, deeper and larger ulcers can develop, causing scarring, stiffness, and possibly narrowing of the bowel, sometimes leading to obstruction **(Shiel *et al*., 2008).**

Cystitis: Inflammation of the bladder, pyelonephritis bacterial infection of the kidneys. Pyelonephritis can be acute or chronic, and it is most often due to the ascent of bacteria from the bladder up the ureters to infect the kidneys. Diagnosis is made via urinalysis, which reveals white blood cells and bacteria in the urine. Usually there is also an increase in circulating white cells in the blood **(Shiel *et al*., 2008).**

Glossary

Diabetes mellitus: A chronic condition associated with abnormally high levels of sugar (glucose) in the blood and urine. Absence of insufficient production of insulin or auto-immune resistance to the pancreatic hormone insulin causes diabetes **(Shiel *et al*., 2008).**

Dysuria: Pain during urination, or difficulty urinating. Dysuria is usually caused by inflammation of the urethra, frequently as a result of infection **(Shiel *et al*., 2008).**

Femoral vein: The large vein in the groin that passes with the femoral artery under the inguinal ligament to enter the abdomen, at which point it becomes the external iliac vein. The femoral vein is a continuation of the popliteal vein, and it carries blood back to the heart from the lower extremities **(Shiel *et al*., 2008).**

Gallstone: A stone of varying size that forms when substances in bile harden. There can be just one large stone, hundreds of tiny stones, or any combination and number. Gallstones can block the normal flow of bile if they lodge in any of the ducts that carry bile from the liver to the small intestine. Bile trapped in these ducts can cause inflammation in the gallbladder, the ducts, or, rarely, the liver **(Shiel *et al*., 2008).**

Gastric ulcer: An ulcer in the lining of the stomach that is corroded by the acidic digestive juices secreted by the stomach cells **(Shiel *et al*., 2008).**

Guillain-Barre syndrome: A rare disorder characterized by a misdirected immune response against the peripheral nervous system associated with progressive symmetrical paralysis and loss of reflexes, usually beginning in the legs **(Shiel *et al*., 2008).**

Hemodialysis: The process of cleansing the blood by passing it through a special machine. Dialysis is necessary when the kidneys are not able to filter the blood. It gives patients with kidney failure a chance to live productive lives. A hemodialysis session generally takes about 4 hours, and patients typically visit a dialysis clinic one to three times per week **(Shiel *et al*., 2008).**

Herniated disk: A disk, situated between two vertebrae that protrudes and tends to press on a nerve root, causing radiating pain. A herniated disk may cause sciatica (pain in the lower back, leg, and behind the knee). Treatment options include use of anti-inflammatory medications, local injection of steroids, and surgical procedures **(Shiel *et al*., 2008).**

Glossary

Hyperglycemia: Elevated blood glucose (sugar). Hyperglycemia is often found in diabetes mellitus **(Shiel *et al*., 2008).**

Hypertrophic heart disease (Cardiomyopathy): A heart defect characterized by increased thickness (hypertrophy) of the wall of the left ventricle, the largest of the four chambers of the heart **(Shiel *et al*., 2008).**

Intestinal anastomosis: A surgical operation to join one part of the intestine to another, after a section has been removed **(Bateman *et al*., 2007).**

Jugular vein, internal: The deeper of the two jugular veins on each side of the neck. The internal jugular vein collects blood from the brain, the outside of the face, and the neck. It runs down the inside of the neck, outside the internal and common carotid arteries, and unites with the subclavian vein to form the innominate vein **(Shiel *et al*., 2008).**

Kidney stone: A stone in the kidney or a stone that originates in the kidney but has passed lower down in the urinary tract. Kidney stones are a common cause of blood in the urine and pain in the abdomen, flank, or groin. The development of kidney stones is related to decreased urine volume or to increased excretion of stone-forming components, such as calcium, oxalate, urate, cystine, xanthine, and phosphate **(Shiel *et al*., 2008).**

Leukemia: Cancer of the blood cells. Strictly speaking, leukemia should refer only to cancer of the white blood cells (leukocytes), but in practice it can apply to malignancy of any cellular element in the blood or bone marrow, as in red cell leukemia (erythroleukemia) **(Shiel *et al*., 2008).**

Meningitis: Inflammation of the meninges, the three membranes that envelop the brain and the spinal cord. Meningitis can be caused by infection by bacteria, viruses, and protozoa. Other causes include cancer (metastasis to the meninges), inflammatory diseases, and drugs. In some cases the cause of meningitis cannot be determined. The treatment depends on the cause of the meningitis **(Shiel *et al*., 2008).**

Multinodullar goiter: A condition in which the thyroid gland contains multiple lumps (nodules) that are overactive and that produce excess thyroid hormones **(Shiel *et al*., 2008).**

Penile area: Pertaining to the penis area **(Shiel *et al*., 2008).**

Glossary

Peritonitis: Inflammation of the peritoneum, the membrane that lines the inner wall of the abdomen and pelvis. Peritonitis can result from infection, as by bacteria or parasites; injury and bleeding; or diseases, such as systemic lupus erythematosus **(Shiel *et al*., 2008).**

Phlebitis: Inflammation of a vein. With phlebitis, there is infiltration of the walls of the vein and, usually, the formation of a clot (thrombus) in the vein (thrombophlebitis). Phlebitis in a leg, for example, causes the leg to swell with fluid (edema). Phlebitis can be superficial and not very serious, or it can be deep and carry the potential for dislodging blood clots to the lungs **(Shiel *et al*., 2008).**

Pulmonary artery: One of the two vessels which are formed as terminal branches of the pulmonary trunk and convey un-aerated blood to the lungs **(Shiel *et al*., 2008).**

Pyloric stenosis: Narrowing (stenosis) of the outlet of the stomach so that food cannot pass easily from it into the duodenum. Pyloric stenosis results in feeding problems and projectile vomiting in infants. The obstruction can be corrected with a relatively simple surgical procedure **(Shiel *et al*., 2008).**

Sepsis: The presence of bacteria (bacteremia), other infectious organisms, or toxins created by infectious organisms in the bloodstream with spread throughout the body. Sepsis may be associated with clinical symptoms of systemic illness, such as fever, chills, malaise, low blood pressure, and mental-status changes. Sepsis can be a serious situation, a life-threatening condition that requires urgent and comprehensive care **(Shiel *et al*., 2008).**

Septic shock: A subset of sepsis in which particularly profound circulatory, cellular, and metabolic abnormalities are associated with a high risk of mortality **(Singer *et al*., 2016).**

Suprapubic area: Above the pubic bone or pubic area **(Bateman *et al*., 2007).**

Stomach cancer: A malignant tumor of the stomach. Gastric cancer can develop in any part of the stomach and can spread from the stomach to other organs **(Shiel *et al*., 2008).**

Tuberculosis: A highly contagious infection that is caused by the bacterium Mycobacterium tuberculosis. Tubercles (tiny lumps) are a characteristic finding in TB. Diagnosis is made via skin test, which if positive is followed by a chest X-ray to determine the status (active or dormant) of the infection. TB is more common in people with immune system problems, including AIDS, than in others **(Shiel *et al*., 2008).**

Glossary

Urethritis: Inflammation of the urethra, the tube that leads from the bladder to the outside of the body. Urethritis can have a number of causes, including irritation and sexually transmitted diseases such as chlamydia. Urethritis is closely associated with bacterial infection of the bladder (cystitis) **(Shiel *et al*., 2008).**

Urinary incontinence (Enuresis): Involuntary urination, which may be caused by a variety of factors, including disorders of the kidneys, bladder, or ureter, and poor control of the muscles that control release of urine **(Shiel *et al*., 2008).**

Vein thrombosis: A blood clot within a deep vein, typically in the thigh or leg. The blood clot (thrombus) can break off as an embolus and make its way to the lung, where it can cause lung problems **(Shiel *et al*., 2008).**

Vena cava, inferior: The large vein that receives blood from the lower extremities, pelvis, and abdomen and then empties that blood into the right atrium of the heart **(Shiel *et al*., 2008).**

Vena cava, superior: The large vein that returns blood to the right atrium of the heart from the head, neck, and both upper limbs. The superior vena cava is located in the middle of the chest and is surrounded by rigid structures and lymph nodes **(Shiel *et al*., 2008).**

Scientific production

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Faculty of **N**atural and **L**ife **S**ciences

Scientific production

Name and First Name of the student: Mrs. Nesrine BOUHROUR

Name and First Name of the thesis supervisor: Prof. Farida BENDALI

International communications

Détection de biofilms sur cathéters veineux prélevés chez des patients hospitalisés au CHU de Bejaia

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Résumé

L'utilisation des cathéters veineux est considérée un grand progrès dans la gestion de nombreuses maladies. En effet, ces dispositifs implantables sont de plus en plus utilisés pour le traitement des déficits fonctionnels dans de nombreux domaines médicaux. Cependant, ces surfaces abiotiques restent sensibles à la colonisation bactérienne ce qui crée un véritable problème de santé publique. La physiopathologie de ces infections est liée initialement à la constitution d'un biofilm sur ces corps étrangers. Dans le but de détecter la présence de biofilms bactériens sur cathéters, 11 cathéters veineux ont été prélevés chez des patients hospitalisés au CHU de Béjaia et ayant séjournés plus de 48 h au niveau de différents services. La présence de ces derniers a pu être mise en évidence après sonication et 80 souches ont été isolées, 32 souches appartenant au genre *Staphylococcus* (40%), 16 au genre *Enterococcus*(20%), 28 à la famille des entérobactéries (35%) et 4 souches non identifiées (5%). Le pouvoir des souches isolées à former des biofilms a été évalué en utilisant des microplaques en polystyrène, ce qui a révélé que 60% d'entre elles étaient fortement formatrices de biofilms. De plus la production du slime, un exopolysaccharide pariétal, permettant l'adhésion bactérienne, a été détectée chez 11,25% des souches. Cette étude, réalisée pour la première fois à Béjaia, met l'accent sur le fort pouvoir colonisateur des souches bactériennes hospitalières, isolées sur cathéters, et le risque infectieux accru qui en découle.

Mots-clés: Adhésion bactérienne, biofilm, cathéters veineux, infections nosocomiales.

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الأكاديمية الجزائرية لجراحي الرأس و الرقبة
ALGERIAN ACADEMY OF HEAD AND NECK SURGEONS

ATTESTATION DE COMMUNICATION

Nous soussignés, attestons que le travail suivant

Détection de biofilms sur cathéters veineux périphériques chez des patients hospitalisés au CHU Bejaia.

a été présenté lors des Journées internationales médicales, portant sur le thème «le risque infectieux en milieu chirurgical», organisées les 30 septembre et 01 octobre 2016 à la faculté de médecine «ZIANA» à Alger

Sous forme d'une communication: ~~orale~~ / affichée

Auteur: N. BOUHROUR

Co-Auteurs: F. BENDALI

Président des journées	président de l'AACTC	présidente du comité d'organisation
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Caractérisation de biofilms bactériens formés sur cathéters et sondes urinaires prélevés au niveau de certains hôpitaux de la wilaya de Bejaia (Algérie)

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Résumé

Les succès de la médecine moderne sont intimement liés à l'utilisation sans cesse croissante de dispositifs médicaux tels que ceux utilisés pour la gestion des fonctions vitales. Cependant, ces surfaces abiotiques, en contact direct avec les patients et l'environnement hospitalier, sont sensibles à la colonisation bactérienne qui représente un sérieux problème de santé publique. En effet, plus de 60% des infections nosocomiales, dans le monde entier, sont imputées aux bactéries formant des biofilms sur ces dispositifs médicaux. Au cours de l'étude menée au niveau de certains hôpitaux de la région de Béjaia (Nord-Est d'Algérie), un total de 7/13 dispositifs prélevés présentait une numération bactérienne supérieure ou égale à 10^3UFC/ml. Un nombre total de 97 souches bactériennes a été isolé et identifié, 80 d'entre elles ont été isolées des cathéters veineux (74,8%) et 17 de sondes urinaires (16%). Ces souches appartenaient aux espèces *Staphylococcus aureus*, *Escherichia coli*, *Klesbsiella sp*, *Enterococcus sp*. L'étude de leur potentiel d'adhésion et de formation de biofilms sur les microplaques de 96 puits a montré que 62,04% d'entre elles sont formatrices de biofilms contre 8,3% qui se sont révélées non adhérentes sous les conditions de l'étude. Ces résultats montrent que ces dispositifs médicaux sont de véritables niches écologiques de bactéries pouvant être un réservoir à partir duquel différentes infections peuvent se développer.

Mots-clés: dispositif médical, colonisation bactérienne, biofilm, infection nosocomiale.

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الجمعية التونسية للبيولوجيا السريرية
Visa n°B12290/1 du 22/7/81

97

Hammamet le 13 Mai 2017

ATTESTATION

Nous attestons que le Poster intitulé :

CARACTÉRISATION DE BIOFILMS BACTÉRIENS FORMÉS SUR CATHÉTERS ET SONDES URINAIRES PRÉLEVÉS AU NIVEAU DE CERTAINS HÔPITAUX DE LA WILAYA DE BEJAIA (ALGÉRIE)

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(1)LABORATOIRE DE MICROBIOLOGIE APPLIQUÉE, FACULTÉ DES SCIENCES DE LA NATURE ET DE LA VIE, UNIVERSITÉ DE BEJAIA

A été présenté lors du Ier Congrès Maghrébin de Biologie Clinique et des XXXI ^èmes JNBC tenues à Hammamet du 11 au 13 Mai 2017

Pour le comité d'organisation
du Ier Congrès Maghrébin de Biologie Clinique
et des XXXI ^èmes JNBC

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Virulence des souches bactériennes isolées de dispositifs médicaux au niveau de deux structures de soins à Béjaia

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Résumé

Le risque des infections nosocomiales est incontournable en particulier avec le recours aux implants médicaux. Ces dispositifs très efficaces mais de nature invasive sont reconnus une importante source de transmission des micro-organismes de l'environnement hospitalier. Ces derniers connus très virulents et l'un de leurs principaux facteurs de virulence est leur capacité à former un biofilm. Le problème majeur avec cette forme de vie est qu'elle confère une résistance importante aux antibiotiques et aux attaques du système immunitaire. Dans cette étude, 108 souches bactériennes ont été isolées de cathéters veineux et de sondes urinaires, utilisés dans deux structures de soins au niveau de la région de Béjaia. La virulence des souches bactériennes isolées de ces dispositifs a été évaluée en utilisant le test d'hémolyse sur une gélose au sang, cela a révélé qu'un taux de 44 % des souches (46/108) présentait une activité hémolytique de type β. Les résultats de la méthode MATH (Microbial Adhesion To Hydrocarbons) ont montré un caractère hydrophobe chez 57,1 % (18/28) des souches testées avec un pourcentage d'hydrophobicité allant de 55 % à 85 %, un caractère prédictif du pouvoir adhésif (première étape d'invasion cellulaire et tissulaire) des souches pathogènes chez l'hôte. Le détachement et la diffusion du biofilm formé dans les dispositifs, implantés durant plus d'une semaine, ont été testés par la technique de contact sur gélose au sang. Cette dernière a montré une bonne diffusion du biofilm au bout de 24 h. Cette étude met l'accent sur la virulence des souches bactériennes hospitalières, isolées sur les dispositifs médicaux.

Mots-clés: dispositif médical, hémolyse, biofilm, hydrophobicité, détachement

ATTESTATION DE COMMUNICATION

A L'occasion de la Première Conférence Internationale d'Infectiologie d'Annaba (CiiA)

Nous attestons par ce document que le **Dr N.Bouhrour** a présenté une communication **affichée** intitulée :

Virulence des souches bactériennes isolées de dispositifs médicaux au niveau de deux structures de soins à Béjaia

(Co-auteur):

F.Bendali

Lors de cette journée, organisée à la faculté de médecine d'Annaba le 27 et 28 octobre 2017

Président du comité d'organisation	Président de la séance	Président du Comité Scientifique
Centre Hospitalier Universitaire d' Annaba Service d'Infectiologie Pr. MEGHADECHA Mohamed Lhadi Maître de Conférences Hospitalo-Universitaire N° 237	Pr [illegible] Sabrina Chef [illegible] - CHU ANNABA-	Pr. M. LAOUAR Clinique des Maladies Infectieuses C.H.U. ANNABA Hopital Universitaire Dr. DORBAN N° 118

PROPRIETES DES SOUCHES BACTERIENNES ISOLEES DES CATHETERS VEINEUX AU NIVEAU DU CHU DE BEJAIA

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Résumé

Introduction

Les biofilms sont des communautés de microorganismes en contact avec une surface. Bien que bénéfiques dans la plupart des environnements, les biofilms bactériens se développant sur des implants ou lors d'infections chroniques constituent des réservoirs de pathogènes à l'origine de nombreuses infections nosocomiales constituant ainsi un motif important de mortalité et de morbidité.

Matériel et méthodes

Dans le but d'étudier les caractéristiques des souches isolées des cathéters veineux, les souches bactériennes ont été isolées selon la méthode de Brun Buisson et identifiées avec des galeries biochimiques classiques. L'hydrophobicité de ces souches a été étudié en utilisant le test de MATH qui consiste à mettre en contact une suspension bactérienne de densité optique connue avec un solvant (xylène) puis mesurer la DO de la phase aqueuse. La virulence a été évaluée en utilisant le test d'hémolyse sur une gélose au sang.

Résultats

Sur les 11 cathéters veineux prélevés chez des patients hospitalisés plus de 48h au CHU de Béjaia, un total de 76 souches ont été isolés. 32 souches appartenant au genre *Staphylococcus* (40%), 16 au genre *Enterococcus* (20%), 28 à la famille des entérobactéries (35%). Les résultats de la méthode de MATH ont montré que le caractère hydrophobe a été observé chez 48% des souches isolées (22/46). Le test d'hémolyse a révélé qu'un taux de 61,80% des souches (47/76) présentaient une activité hémolytique de type β.

Discussion et Conclusion

Cette étude, réalisée pour la première fois à Bejaïa, met l'accent sur le fort pouvoir colonisateur des souches bactériennes hospitalières, isolées sur cathéters, et le risque infectieux accru qui en découle ainsi que sur le caractère hydrophobe des souches isolées. Ce dernier étant un caractère prédictif du pouvoir adhésif des souches pathogènes chez l'hôte.

Mots clés: biofilm, adhésion bactérienne, dispositif médical, virulence.

CHU DE BEJAIA

Service des Maladies Infectieuses
Centre de référenece IST/VIC/SIDA

2èmes
JOURNÉES INTERNATIONALES
D'INFECTIOLOGIE DE BÉJAIA
28 ET 29 AVRIL 2018

جامعة بجاية
Tasdawit n Bgayet
Université de Béjaïa
Faculté de Médecine

Attestation

Mr/ Mme : BOUHROUR. N., BENDALI. F. a présenté une communication affichée intitulée :

« Propriétés des souches bactériennes isolées des cathéters veineux au niveau du CHU de Béjaia »

Président de la séance

Président du comité d'organisation

Pr. NOUASRIA Boubaker
Chef de Ser. des Maladies Infectieuses

Président du comité scientifique

Virulence des souches bactériennes isolées de sondes urinaires prélevées au niveau de certaines structures de soins de la wilaya de Bejaia

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Résumé

Les succès de la médecine moderne sont intimement liés à l'utilisation sans cesse croissante de dispositifs biomédicaux tels que ceux utilisés pour la gestion des fonctions vitales. Cependant, ces surfaces abiotiques restent sensibles à la colonisation bactérienne ce qui crée un véritable problème de santé publique. La physiopathologie de ces infections est liée initialement à la constitution d'un biofilm sur ces corps étrangers. Le problème majeur avec cette forme de vie est qu'elle confère une résistance importante aux antibiotiques et aux attaques du système immunitaire. Un total de 35 souches bactériennes ont été isolées. 13 souches appartenant à la famille des entérobactéries (37%), 11 souches au genre *Staphylococcus* (32%) et 11 souches au genre *Enterococcus* (32%). Le pouvoir des souches isolées à former des biofilms a été évalué en utilisant des microplaques en polystyrène, ce qui a révélé que 52% d'entre elles étaient fortement formatrices de biofilms. La virulence des souches bactériennes isolées de ces dispositifs a été étudiée en utilisant le test d'hémolyse sur une gélose au sang à 5%, cela a montré qu'un taux de 43% des souches (16/35) présentait une activité hémolytique complète du type B. Ces résultats montrent que ces sondes urinaires sont de véritables niches écologiques de bactéries pouvant être à l'origine d'infections nosocomiales causant un véritable problème pour la santé publique.

Mots clés: Souche bactérienne, biofilm, adhésion, sonde urinaire, virulence.

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ATTESTATION*de* COMMUNICATION

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BOUHROUR NESRINE

A PARTICIPE AU 7ème CONGRES DE LA BIOLOGIE MEDICALE ET MEDECINE DE LABORATOIRE ORGANISE LE 6 ET 7 MAI 2018 A ALGER PAR UNE COMMUNICATION AFFICHEE, INTITULEE :

VIRULENCE DES SOUCHES BACTERIENNES ISOLEES DE SONDES URINAIRES PRELEVEES AU NIVEAU DE CERTAINES STRUCTURES DE SOINS DE LA WILAYA DE BEJAIA

Co-auteurs : BENDALI F

Président du comité scientifique

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Activité antimicrobienne du peptide SAAP-148, un dérivé d'origine humaine, contre les biofilms formés sur cathéters

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Résumé

L'émergence de la résistance aux antibiotiques, en milieu hospitalier, représente une préoccupationmondialemajeureetelleestaggravéeparlaformationdebiofilms.Ellemeten péril la santé publique par l'augmentation du taux de mortalité et de morbidité chez les patients mais aussi des couts d'hospitalisation effroyables. Les peptides antimicrobiens(PAM) sont considérés comme des alternatives potentielles aux antibiotiques conventionnels. Les peptides antimicrobiens sont des molécules, d'origine microbienne, végétale ou animale, de nature cationique, amphipathiques, composées de 11 à 50 acides aminés avec un large spectre d'activités (bactéries Gram négatives et positives), y compris des souches résistantes aux antibiotiques. Ils agissent par perméabilisation des membranes cellulaires, ce qui entraîne l'apparition de pores conduisant ainsi à une lyse bactérienne et jouent un rôle crucial dans l'immunité humaine. Des études récentes ont rapporté l'effet anti-biofilm de ces peptides montrant ainsi une option thérapeutique prometteuse contre les infections liées aux biofilms. Au cours de cette étude, quatre souches originaires de cathéters, isolées à l'hôpital Khalil Amrane(Bejaia) et appartenant aux bactéries Gram négatives (une souche d'*Escherichia coli*, deux souches de *Klebsiella pneumoniae* et une souche d'*Acinetobacter baumannii*) résistantes à deux antibiotiques et plus ont été étudié pour leur capacité à former des biofilms sur microplaque en polystyrène. De plus, l'activité antibactérienne et anti-biofilm du peptide SAAP-148 (un dérivé du peptide humain cathelicidine LL-37) ont été également testées contre ces souches bactériennes. Leur potentiel d'adhésion sur microplaques de 96 puits a montré que toutes les souches étaient fortement formatrices de biofilm. Pour l'activité antibactérienne, le peptide SAAP-148 a tué les souches sélectionnées de manière dose-dépendante après 4 h d'exposition. Ce peptide a également réduit le nombre de bactéries dans des biofilms de 24 h des quatre souches sur des disques en silicone. Cette étude nous a permis de souligner la forte capacité d'adhésion des souches isolées mais aussi que le peptide SAAP-148 semble être prometteur pour l'éradication des biofilms.

Mots clés: Biofilm, antibio-résistance, peptide SAAP-148, cathéter, bactéries Gram négatives.

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Centre de Recherche en Biotechnologie C.R.Bt, Constantine

C.R. Bt

Attestation de communication

Le comité d'organisation du **Séminaire National Hybride « La Biotechnologie au Service de l'Innovation » (BSI- 21)**, organisé par la Faculté des Sciences de la Nature et de la Vie (FSNV) et le Centre de Recherche en Biotechnologie (C.R.B.t) à l'**université de Bejaia les 08 et 09 Décembre 2021**, atteste que Mme **BOUHROUR Nesrine**, Université de Bejaia a présenté une communication **affichée** intitulée : **«Activité antimicrobienne du peptide SAAP-148, un dérivé d'origine humaine, contre les biofilms formés sur cathéters.».**

Co-auteurs : Nibbering Peter et Bendali Farida.

Présidente du Séminaire
Pr BENDALI Farida

Séminaire National Hybride « La Biotechnologie au service de l'innovation »
La Présidente du Séminaire

Doyen de la Faculté
Pr KATI Djamel Edine

العميد
الأستاذ كاتي جمال الدين

Directeur du C.R.Bt
Pr AZIOUNE Ammar

Directeur du Centre de Recherche en Biotechnologie
Dr. Ammar AZIOUNE

Medical devices associated biofilms and novel approaches to combat them

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Abstract

The medical devices are commonly used in hospital settings and are indispensable for the treatment and care of patients in healthcare structures. Unfortunately, their inappropriateuseisfrequentandcanincreasetheriskofbacterialinfections.Themost feared complications of medical devices are catheter-related bloodstream infections and Catheter-associated urinary tract infections. Biofilm formation is the key element in the pathogenicity of catheter infections. Indeed, these devices represent a perfect surface for bacterial attachment and biofilm formation. Novel effective agents are urgently needed. Antimicrobial peptides, which are molecules of the first line of defense against infections, are considered as promising candidates. Among them, SAAP-148, a LL-37-based synthetic antimicrobial and anti-biofilm peptide which was developed using random amino acid substitutions in the C-terminal part of LL-37. Recently, using computer-assisted deep learning discovery approaches, halicin which was identified as the first-in-class novel small molecule, constitute also a promising agent against these infections. In this study, we evaluated the antibacterial, anti-biofilm, and anti-persister activities of SAAP-148 and halicin on Gram-negative bacteria (*E. coli*, *K. pneumoniae* and *A. baumannii*) colonizing catheters originating from Bejaia hospital and selected based on their resistance to at least three antibiotic classes and biofilm formation potential. The bactericidal activity of SAAP-148 was 4-8 fold reduced in urine and plasma compared to buffered saline, whereas halicin‘s efficacyin urine was approximately4-fold less than in buffered saline. SAAP-148 and halicin reduced the number of bacteria within 24 hrs biofilms on silicone elastomer discs. Both agents eliminated persisters originating from antibiotics-exposed 7 days biofilms; halicin being less effective than SAAP-148. A synergistic effect of these agents on the strains; except for *A. baumannii*; was observed. In conclusion, SAAP-148 and halicin are considered as promising agents to combat bacterial colonizing catheters.

Keywords: Medical devices, biofilm, antibiotic resistance, Gram-negative bacteria, SAAP-148, halicin.

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International Conference on Applied Microbiology and Biotechnology
1st ICAMB-2024

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Certificate of Participation

The Scientific Committee of the First International Conference on Applied Microbiology and Biotechnology (ICAMB-2024) held in Bejaia, Algeria on the 16th and 17th of October 2024 certifies that :

Nesrine BOUHROUR

Has participated actively to the conference with an **ORAL COMMUNICATION** titled :

Medical devices associated biofilms and a novel approach to combat them

Co-authors: Peter Nibbering, Farida Bendali

President of the Conference
Prof. Mouloud KECHA

ICAMB-2024

President of the organizing Committee
Prof. Farida BENDALI

National communications

Les biofilms à Staphylocoques source d'infections nosocomiales

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Résumé

Les infections nosocomiales représentent un énorme problème de santé publique universel du fait de leur fréquence, leur gravité et leur coût socio-économique. Elles touchent majoritairement les personnes légèrement ou fortement immunodéprimées et impliquent souvent des bactéries commensales comme *Staphylococcus epidermidis*, *Staphyloccocus aureus*… Ces dernières sont des bactéries redoutables, considérées comme l'une des causes principales de ces infections notamment celles liées aux dispositifs médicaux. Sur ces derniers, la constitution d'un biofilm, en synthétisant une matrice extracellulaire polysaccharidique, leur confère non seulement une résistance importante contre l'arsenal thérapeutique mais aussi aux défenses du système immunitaire. Au cours de l'étude menée au niveau de deux hôpitaux de la région de Bejaïa, 47 souches de *Staphylococcus* spp. ont été isolées des dispositifs médicaux dont 26 étaient présumés appartenir à *Staphylococcus aureus*. La capacité de ces souches à former un biofilm a été étudiée en utilisant la méthode d'adhésion sur microplaque en polystyrène montrant un taux d'adhésion de 77%. Ensuite, leur virulence a été évaluée en effectuant le test d'hémolyse sur une gélose au sang. Ceci a révélé que 42 souches étaient hémolytiques. Cette étude a permis de souligner le fort pouvoir d'adhérence des souches de staphylocoques isolées des structures de soins.

Mots-clés: *Staphylococcus*spp., dispositif médical, colonisation bactérienne, biofilm, infection nosocomiale.

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Attestation de communication

Le comité d'organisation des **4èmes Journées des Sciences de la Nature et de la Vie (JSNV-4)**, organisé par la faculté des Sciences de la Nature et de la Vie à l'université de Bejaia les **03 et 04 Octobre 2018**, atteste que Mme/Melle/Mr **BOUHROUR Nesrine** a présenté une communication **Affichée** intitulée :

« Les biofilms à staphylocoques source d'infections nosocomiales »

Co-auteurs: **OUARABI Liza et BENDALI Farida**

Présidente du comité d'organisation
Pr BENDALI F.

Doyen de la Faculté
Pr ATMANI D.

الأستاذ: عتماني جبار

Facteurs de virulence de souches de *Klebsiella pneumoniae* isolées des sondes urinaires

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Résumé

Klebsiella pneumoniae est un agent pathogène opportuniste et ubiquitaire. Il est considéré comme étant une véritable menace directe pour la santé humaine du fait de sa capacité à résister à de nombreux antibiotiques et son implication dans la propagation de gènes de résistance aux antibiotiques. D'autre part, sa grande capacité à coloniser des dispositifs médicaux en produisant une épaisse couche polysaccharidique qui aide l'organisme à se fixer aux surfaces abiotiques appelé «biofilm» est également un facteur crucial contribuant au développement des infections nosocomiales, dont les infections associées au tractus urinaire, augmentant ainsi le taux de mortalité. Dans cette étude, la capacité d'adhésion de six souches de *K. pneumoniae* sur microplaques de 96 puits a révélé que 50% d'entres elles étaient fortement formatrices de biofilm. L'hydrophobicité de la surface bactérienne a montré que les souches EN34 et EN35 étaient moyennement hydrophobes avec des taux de 30,82% et 40,51% respectivement. L'auto-agrégation des souches a montré des pourcentages allant de 9,68% jusqu'à 27,48%. Leur profil de résistance aux antibiotiques testés selon la méthode de diffusion sur disques était comme suit : Amoxicilline+acide clavulanique, cefepime et tétracycline (100%) ; cefotaxime et ceftazidime (83,33%); gentamycine (50%); cefoxitine, imipinem, ciprofloxacine, acide nalidixique et cotrimoxazole (0%). De plus, des images de synergie ont été observées chez deux souches indiquant probablement la production d'une BLSE. Cette étude souligne que ces souches possèdent diverses propriétés telles que la formation de biofilm et la résistance aux antibiotiques qui semblent être des facteurs considérables dans leur pathogénicité.

Mots-clés: *Klebsiella pneumoniae*, biofilm, sonde urinaire, résistance aux antibiotiques, infection nosocomiale.

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ATTESTATION DE COMMUNICATION

Le comité d'organisation de la 1ère journée doctorale des Sciences de la Nature et de la Vie, organisée par la Faculté des Sciences de la Nature et de la Vie à l'université de Bejaia le 16.12.2019

atteste que :

Mr/Mme/Mlle :**BOUHROUR Nesrine**

A présenté une communication **Poster** intitulée : « **Facteurs de virulence de souches de *Klebsiella pneumoniae* isolées des sondes urinaires».**

Co-auteurs : **BENDALI F.**

Le président du comité d'organisation

Dr AMIR Nadir

Le doyen de la faculté SNV

العميد
الأستاذ : لحشماني جبار

CARACTERISTIQUES DES SOUCHES D'*ENTEROCOCCUS* SPP. ISOLEES CHEZ DES PATIENTS CATHETERISES AU NIVEAU DE L'HOPITAL KHALIL AMRANE (BEJAIA)

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Résumé

La résistance aux antibiotiques représente une préoccupation importante dans les infections nosocomiales et est aggravée par la formation de biofilms, communautés caractérisées par une résistance 10 à 1000 fois plus que les cellules planctoniques. Les espèces du genre *Enterococcus* sont des agents pathogènes opportunistes pouvant survivre dans l'environnement hospitalier. Parmi elles, *Enterococcus faecalis* est l'espèce la plus communément impliquée dans les infections cliniques à entérocoques. Elle forme facilement un biofilm sur les dispositifs médicaux tels que les cathéters veineux et les sondes urinaires, ce quicontribue à son pouvoir pathogène et augmente ainsi la gravité des infections. Au cours de cette étude, 33 souches d'*Enterococcus* spp. ont été isolées, dont 17 souches originaires de cathéters veineux et 16 souches de sondes urinaires. Leur potentiel d'adhésion sur microplaques de 96 puits a montré que toutes les souches étaient fortement formatrices de biofilm. Le test d'hémolyse a révélé que seulement 18,18 % des souches présentaient une activité hémolytique de type β contre un taux de 81,8 % de souches non hémolytiques. Pour la sensibilité aux antibiotiques, les souches étaient résistantes à la Vancomycine et à la Tétracycline (87,9 %), à la Ciprofloxacine (84,8 %), à l'Erythromycine (81,8 %), à la Rifampicine (0,2 %) et au Co-trimoxazole (0,03 %). Aucune résistance n'a été notée à l'égard de l'Imipenème. Cette étude, réalisée pour la première fois dans la région de Béjaia nous a permis de souligner la forte capacité d'adhésion des souches d'entérocoques isolées et leur taux de résistance aux différents antibiotiques testés. La surveillance des infections liées aux dispositifs médicaux est devenue un objectif primordial de tout programme de lutte contre les infections nosocomiales.

Mots clés: biofilm, infection nosocomiale, *Enterococcus faecalis*, cathétérisme, résistance.

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SNASE 2019

Attestation de communication

Le comité d'organisation du **séminaire national « Alimentation, Santé et Environnement : Avancées et Perspectives »**, organisé par la Faculté des Sciences de la Nature et de la Vie à l'université de Bejaia les **25 et 26 Septembre 2019**, atteste que Melle **BOUHROUR Nesrine** a présenté une communication **Poster** intitulée :

«Caractéristiques des souches d'*Enterococcus* spp. isolées chez des patients cathétérisés au niveau de l'Hôpital Khalil Amrane (Bejaia)»

Co-auteurs : **OUARABI Liza & BENDALI Farida.**

Présidente du comité d'organisation
Pr. BENDALI F.

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Caractérisation des souches d'*Acinetobacter* spp. productrices de biofilms isolées sur cathéters veineux périphériques

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Résumé

Acinetobacter spp. sont des agents pathogènes opportunistes importants responsables de plusieurs infections nosocomiales telles que la pneumonie associée à la ventilation, méningite, bactériémie, infections des plaies et infections sur cathéters veineux. C'est devenu un problème sérieux dans le traitement des infections à *Acinetobacter* spp. en raison de leur résistance hautement élevé à presque tous les antibiotiques connus et aux réponses immunitaires de l'hôte. De plus, Ces souches multi-résistantes ont une incroyable capacité de survivre et de persister sur différences surfaces biotiques et abiotiques due à la formation de biofilms. La production de biofilms chez *Acinetobacter* spp. est un mécanisme complexe régulé par différents facteurs de virulence incluant la protéine de membrane externe A(OmpA), la protéine associée au biofilm (Bap) et les systemes pili (Csu) qui permettent l'adhésion aux surfaces, la colonisation, l'invasion et la production de biofilms. Au cours de notre étude menée dans certains services de l'hopital Khalil Amrane de Bejaia. Un total de six souches d'*Acinetobacter* spp. a été isolé et identifié à partir de cathéters veineux périphériques dont cinq souches appartenaient à l'espèce *Acinetobacter baumannii* et une souche à l'espèce *Acinetobacter lwoffi*. L'étude de leur capacité à former de biofilms sur les microplaques de 96 puits a montré que 83.33% d'entre elles sont productrices de biofilms contre 16.7% qui se sont révélées non adhérentes sous les conditions de l'étude. Selon les résultats de la PCR, toutes les souches d'*Acinetobacter* spp. possédaient le gène *ompA*, 5/6 et 4/6 des souches étaient positives pour les genes *csu* et *bap*, respectivement. Pour la sensibilité aux antibiotiques, les souches étaient résistantes à la ceftazidime (83.33%), cefotaxime (83.33%), cefepime, imipineme, tétracycline, ciprofloxacine et cotrimoxazole (100%). Ces résultats révèlent la forte capacité de production de biofilm chez *Acinetobacter* spp., avec une prévalence élevée de gènes liés au biofilm. Des mesures de surveillance et de contrôle appropriées soient essentielles pour prévenir leur émergence et leur transmission dans notre pays.

Mots- clés: Cathéter veineux, *Acinetobacter* spp., Adhesion bactérienne, biofilm,gène de virulence, infection nosocomiale.

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Attestation de participation

Le comité d'organisation de la 1ère journée doctorale de Microbiologie Appliquée (JD-LMA), tenue à l'auditorium de l'université A.MIRA de BEJAIA, le 05 Décembre 2021, atteste que:

Melle BOUHROUR Nesrine

A participé activement à la journée et a présenté une communication **Affichée** intitulée :

Caractérisation des souches d'*Acinetobacter* spp. productrices de biofilms isolées sur cathéters veineux périphériques

Co-auteurs: BENDALI.F.

Bejaia Le : 05/ 11/ 2021

Le comité scientifique

كمشة مولود

Caractérisation des bactéries pathogènes isolées de différents dispositifs médicaux prélevés chez des patients hospitalisés

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Résumé

Les cathéters sont essentiels dans la gestion d'une série de scénarios cliniques, tels que l'administration de chimiothérapie, d'antibiotiques et de nutrition parentérale. Malheureusement, leur utilisation est compliquée par leur propension à être colonisée par des bactéries, ce qui peut entraîner des infections graves, telles que des infections sanguines et urinaires récurrentes, qui affectent considérablement la durée des séjours à l'hôpital, les taux de mortalité et les coûts. Le traitement de ces patients porteurs d'un cathéter infecté implique souvent le retrait du dispositif infecté, suivi d'une antibiothérapie intensive. L'échec de ce traitement est dû à l'émergence de souches résistantes aux antimicrobiens et/ou à la formation de biofilms et de bactéries persistantes. Un total de 219 souches bactériennes ont été isolées à partir de 33 dispositifs médicaux (cathéter intraveineux, sonde urinaire et lame intra-abdominale) collectés chez des patients hospitalisés au niveau des différentes structures de soins de la wilaya de Bejaia (Nord-Est d'Algérie). Les souches les plus isolées étaient *Staphylococcus* spp. (24 *S. aureus* et 119 *Staphylococcus* à coagulase négative) suivis par *Enterobacteriaceae* (31 *E. coli* et 6 *K. pneumoniae*), 33 *En. faecalis* et *Acinetobacter* spp. (5 *A. baumannii* et 1 *A. lwoffii*). Ces souches ont été étudiées pour leur capacité à former des biofilms par les méthodes de la plaque de culture tissulaire et de la gélose au rouge Congo. 85,84 % et 10 % des souches étaient fortement formatrices de biofilms et de slime, respectivement. De plus, les souches possédaient plusieurs gènes de virulence (*fimH*, *mrkD*, *fimH-1*, *ompA*, *csuE*, *icaA*, *icaD*, *esp* et asa1) impliqués dans l'adhésion et la formation de biofilm. Ces résultats montrent le fort pouvoir pathogène de ces souches bactériennes isolées des dispositifs médicaux soulignant ainsi la nécessité de surveiller ces infections nosocomiales en Algérie.

Motsclés: Dispositif médical, biofilm, pathogène, infection, virulence.

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Université Abderrahmane MIRA Bejaia
Faculté des Sciences de la Nature et de la Vie

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ATTESTATION

SEPTIÈMES JOURNÉES SNV

10 et 11 Décembre, 2024

Le comité d'organisation des 7èmes Journées des Sciences de la Nature et de la Vie, atteste que :

Nesrine BOUHROUR

Co-auteurs : Farida BENDALI et Peter Nibbering

a participé avec une Communication Affichée intitulée : Caractérisation des bactéries pathogènes isolées de différents dispositifs médicaux prélevés chez des patients hospitalisés.

Pr. BETTACHE A.	Pr. DJOUDAD-KADJI H.	Pr. HADDADI-GUEMGHAR H.
Doyen de la FSNV	Présidente du Comité d'Organisation	Présidente du Comité Scientifique

عز الدين بطاش
عميد كلية علوم الطبيعة و الحياة

Scientific articles:

Bouhrour, N.; Bendali, F.Venous Catheters Infections Associated to *Staphylococcus* Biofilms: Worldwide Problem of the 21st Century. J. *Immunol.Res.Ther*.**2019**; 2,115.

Lien: https://scientificliterature.org/Immunology/Immunology-19-115.pdf

Bouhrour, N.; van der Reijden, T.J.K.; Voet, M.M.; Schonkeren-Ravensbergen, B.; Cordfunke, R.A.; Drijfhout, J.W.; Bendali, F.; Nibbering, P.H. Novel Antibacterial Agents SAAP-148 and Halicin Combat Gram-Negative Bacteria Colonizing Catheters. *Antibiotics* **2023**, *12*, 1743. https://doi.org/10.3390/antibiotics12121743.

Lien:https://www.mdpi.com/2079-6382/12/12/1743

Bouhrour, N.; Nibbering,P.H.; Bendali,F. Medical Device-Associated Biofilm Infections and Multidrug-Resistant Pathogens. *Pathogens* **2024**, *13*, 393. https://doi.org/10.3390/pathogens13050393.

Lien: https://www.mdpi.com/2076-0817/13/5/393

Immunology Research And Therapy Journal

SCIENTIFIC LITERATURE

Special Issue Article" Staphylococcus" Review Article

Venous Catheters Infections Associated to *Staphylococcus* Biofilms: Worldwide Problem of the 21st Century

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ABSTRACT

Venous catheters are increasingly used in medical care. However, these devices are easily colonized by microorganisms. *Staphylococcus* spp. are the dominant species in catheter-related infections because of their ability to form biofilms on their surfaces, giving them resistance to antimicrobial agents and leading to recalcitrant chronic infections and therapeutic failure. In addition, the emergence of methicillin-resistant Staphylococci is a global scourge for public health. In this review, an update of our knowledge on staphylococcal biofilms, their formation steps, regulation, resistance to methicillin, their involvements in the worldwide emergence of venous catheters-related infections and treatment strategies for biofilm prevention and eradication have been reported.

INTRODUCTION

Several bacterial species colonize the human body and could be both useful and dangerous for health. Among them, *Staphylococcus aureus*, which is a commensal of the skin, is considered a fearsome pathogen implicated in nosocomial infections [1], especially those related to medical devices such as venous catheters (Figure 1). The emergence of Methicillin-Resistant *S. aureus* (MRSA) is mainly the major clinical problem in hospitals [2]. Its ability to produce biofilm was considered a major virulence factor of pathogenesis [3] causing the failure of these devices [4]. Bacterial colonization on medical devices is the first step in the development of chronic infections and their persistence [5]. Biofilms consist of one or more species adhering to a biotic or abiotic surface and surrounded by an extracellular polysaccharide matrix produced by them [6].In recent years, it has become apparent that their importance in the medical community is crucial. Indeed, what makes these biofilms of paramount importance is their ability to resist antibiotic treatment and immune system attacks leading to therapeutic failure and persistence of infections [7]. In addition, biofilms are involved not only in the colonization of surfaces but they represent a reservoir of dissemination of bacteria in the body [8]. Venous catheters are often used for infusion, administration of drug and nutrition. However, the inappropriate use of these medical devices can lead to catheter-related infections, increasing morbidity and mortality, length of hospital stay and the cost of the care [9].

***Staphylococcus* species and their virulence factors involved in biofilm formation**

Staphylococcus spp. are Gram-positive cocci, non-motile, non-sporulated, aero-anaerobic facultative, classified into two main groups, coagulase positive

staphylococci and, most of them, coagulase negative staphylococci "CNS" [10] and are part of the natural flora of the skin and mucous membranes of humans [11].

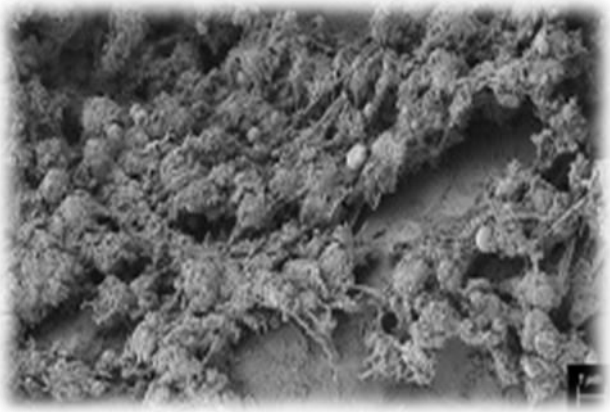


Figure 1: Scanning electron microscopy (SEM) examination of MRSA biofilm formation on central venous catheter implanted in rats [137].

Staphylococcus spp. are opportunistic human pathogens with remarkable adaptability [12]. *Staphylococcus epidermidis* and *S. aureus* are the main cause of catheters-related infections due to their virulence factors, mainly biofilm formation [11]. In recent years, the clinical emergence of methicillin-resistant *Staphylococcus* strains has created many therapeutic challenges for microbiologists and clinicians [10]. When intravenous catheters are implanted in patients, they are rapidly coated with body fluid proteins, such as fibrinogen, fibronectin and collagen, facilitating adhesion of *Staphylococcus* cells expressing on their cell wall surface proteins called the Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMS), that bind specifically to the above mentioned proteins [13,14]. The production of Polysaccharide Intercellular Adhesin (PIA) by enzymes encoded by *icaABCD* operon and composed of linear N-acetyl glucosamine residues linked to β-1-6 is the main mechanism involved in biofilm formation that may contribute to the persistence of infections [3,15]. This operon is composed of four genes encoding four proteins that are involved sequentially in: synthesis of a poly-N-acetylglucosamine polymer *(icaA)* in the presence of *icaD*, translocation of this polymer to the bacterial surface *(icaC)*, deacetylation of the polymer to allow it to attach to the surface *(icaB)*. However, the *icaR* gene plays the role of regulator of this operon [16]. This mechanism has been described for the first time in *S. epidermidis* [17]. These authors demonstrated that mutation of the transposon at the *ica* operon level of *S. epidermidis* altered PIA production and biofilm formation. The study of Mirzaee *et al.* [18] reported that 12/31 biofilm producers *S. aureus* strains (38.7%) were *ica*A and *ica*D positive. More recently, Manandhan *et al.* [19] reported that 22.9% of the biofilm producers *Staphylococcus* strains possessed the *ica*AD genes. However, some Staphylococci species that do not express PIA can also produce biofilm. For example, Kord *et al.* [20] have shown that 4.8% of *S. epidermidis* strains were biofilm producers despite the absence of the *ica* genes. This suggests that the ability to produce biofilm is not always related to the expression of the *ica* genes and may involves other adhesins in cell aggregation and adhesion [15,21] such as biofilm-associated proteins (Bap) [22], Autolysins [23], fibronectin binding proteins (FnBP) [24], collagen binding proteins (Can) [25], elastin binding proteins (EbpS) [26], Protein A [27] and α-enolase (Eno) [28]. Adhesion factors of *Staphylococcus* species and their role in pathogenesis are represented in table 1.

Regulation of *Staphylococcus* biofilm formation

In Staphylococci, biofilm formation is controlled by two systems. The first is named accessory gene regulator (agr). This system produces auto-inducing peptides that regulate the expression of virulence factors as a function of bacterial density [29,30]. Each *Staphylococcus* species contains an agr operon and a specific auto-inducer peptide that vary in length and sequence. In addition, all these peptides contain a thiolactone nucleus and an N-terminal extremity [31]. The proteins that intervene in the *quorum sensing* are regulated by the *agr*BDCA operon. This operon encodes a two-component system, AgrC and AgrA that will detect the auto-inducing peptides produced by the AgrB and AgrD proteins [29]. When the bacterial density is high, the self-inducing peptide activates the AgrC histidine kinase which in turn phosphorylates the AgrA response regulator. The latter binds the promoter P2 which activates RNAII which in turn encodes the proteins AgrA, AgrB, AgrC and AgrD and to the promoter P3 which activates the transcription of the RNAIII which in turn activates the expression of the genes [32,33]. The second regulator system is called staphylococcal accessory regulator A *(sarA)* that regulates also other virulence factors by

modulation of *agr* expression [34]. Sigma B *(sigB)* is an alternative factor that regulates the expression of survival factors against oxidative stress, antibiotics, temperature variations and other environmental stress [35].

Table 1: Adhesion factors involved in biofilm formation in Staphylococcus spp.

Factors		Gene	Function	Reference
Adhesins	biofilm associated protein	*bap*	Adhesin involved in attachment, adhesion on surfaces, and biofilm formation.	Seng *et al.*, [3].
	Autolysin/adhesion	*atLE*	Peptidoglycan hydrolase that plays a role in the degradation of the bacterial wall facilitates adhesion to polystyrene surfaces and biofilm production.	Porayath *et al.*, [23].
	Fibronectin binding protein	*fnBP A and B*	This adhesin is involved in primary adhesion of staphylococcal strains to surfaces and also in aggregation and intercellular adhesion.	Kırmusaoğlu, [149].
	Collagen binding protein	*can*	This extracellular protein of *S. aureus* binds to collagen and is involved in bacterial adhesion and immune evasion.	Herman-Bausier *et al.*, [25].
	Elastin binding protein	*ebpS*	Protein with a molecular weight of 25 kDa,present on the surface of *S. aureus* cells and promotes the colonization of mammalian tissues, facilitating pathogenesis.	Downer *et al.*, [150] ; Kot *et al.*, [28].
	Protein A	*spa*	This protein expressed by all strains of *S. aureus* is able to block opsonophagocytosis by binding to Fcγ in the presence of antibodies and plays an important role in the immune evasion and pathogenicity of Staphylococci.	Hong *et al.*, [27] ; Lacey *et al.*, [151].
	α-enolase	*eno*	This protein expressed on the surface of Staphylococci is able to bind to laminin thus allowing adhesion to the extracellular matrix and tissue colonization of the host.	Kot *et al.*, [28].

Staphylococcal enzymes involved in auto-biofilm dispersion

Biofilm dispersion is caused by the production of extracellular enzymes and surfactants that degrade the polymeric matrix [36]. Among these enzymes, proteases are mainly distinguished because of their ability to degrade extracellular proteins, major components of the matrix [37]. These molecules, regulated by *agr*, *sar*A and *sig*B systems, are considered among the most important virulence factors involved in the development of biofilms [38]. In addition, over-expression of proteases regulated by *sar*A and *sig*B systems has been reported to improve planktonic growth suggesting an inverse relationship between biofilm formation and expression of these enzymes [39]. *S. aureus* express one metalloprotease (aureolysinAur), two cysteine proteases (staphopains SspB and ScpA) and seven serine proteases (SspA and V8) [36]. However, *S. epidermidis* produces fewer proteases such as the metalloprotease SepA, the serine protease Espand the cysteine protease EcpA [38]. These enzymes are involved on the one hand in the acquisition of peptide nutrients but also in the evasion of the immune system of the host by interfering with neutrophils [40] and degradation of specific proteins composing the biofilm matrix in order to release planktonic bacteria. For example, V8 protease has the ability to cleave fibronectin binding protein and Bap. UreolysinAur is able to degrade clumping factor b and Bap. Nevertheless, staphopains A and B allow dispersion without specific protein cleavage [37]. Contrariwise, the metalloprotease SepA of *S. epidermidis* is involved in the intercellular accumulation stage where it cleaves the AtlE autolysin which will release the DNA necessary for the formation of the matrix "eDNA" [38]. Nucleases are the second class of enzymes produced by *Staphylococcus* spp. that degrade extracellular DNA (eDNA). In *S. aureus*, the system SaeRS is involved in nucleases regulation, which protects the bacteria against neutrophils released by the host during their detachment from the biofilm [41]. Nuclease (Nuc) and nuclease 2 (Nuc2) are the most common enzymes produced by *S. aureus* [36]. The implication of nucleases during biofilm dispersion has been demonstrated using nuclease deficient *S. aureus* strains that inhibited the liberation of planktonic bacteria [42]. PSMs (phenol soluble modulins) are peptide surfactants produced by *S. aureus* and *S. epidermidis*, and are also involved in the dispersion of biofilms. Their expression is controlled by the *agr* system [43]. PSMδ and PSMβ are the effector molecules produced by *S. aureus* and *S. epidermidis* respectively [39,44] showed that anti-PSMβ factors inhibited *S. epidermidis* strains spread from catheters, using mouse model of medical device related infection, concluding the importance of these surfactants in biofilm dispersal. Nonetheless, these surfactants are also involved during the stage of maturation of the biofilm and play

a major role in the formation of the three-dimensional structure (thickness, volume, roughness) [45].

Mechanism of *Staphylococcus* biofilm formation on intravenous catheters

Biofilm formation in *S. aureus* occurs in three stages: adhesion/attachment, maturation and dispersion [46]. After insertion of the catheter, the initial adhesion of the bacteria is done through the van der walls, electrostatic and hydrophobic interactions [46]. Once the surface of these devices is covered with host proteins (collagen, fibrinogen, fibronectin, vitronectin), the bacteria express genes that encode the MSCRAMMs that will interact with them [47]. The irreversible adhesion to the catheter is due to the production of the extracellular matrix [48]. Once the attachment is over, the *quorum sensing* regulator *agr* represses the expression of these factors which are useless [39]. During the maturation stage, the bacteria will become intergraded and multiply, producing a heterogeneous exopolysaccharide matrix composed of proteins, eDNA and teichoic acids, thus forming a three-dimensional structure also called "mushroom". Simultaneously, an intercellular aggregation took place after the PIA synthesis [47]. The bacteria organize themselves in the biofilm according to their metabolism and respiratory type. Thus, strict anaerobic bacteria will live at the bottom of the biofilm [48]. In addition, bacterial populations characterized by a very slow growth called “dormant or persistent cells” are observed during the exponential and stationary growth phases. These cells are endowed with a high resistance to antibiotics which can be the cause of the persistence of infections [49].Formation of biofilm is usually followed by a dispersal step. The latter is characterized by the release of planktonic bacteria in order to infect other sites and to form new biofilms [48]. This step can be triggered either by external physical forces (shearing fluids) or by the production of extracellular enzymes such as proteases, nucleases and PSM [37] as described above.

Methicillin-resistant *Staphylococcus* spp. (MRSA)

Staphylococcus aureus is one of the bacteria with an incredible ability to acquire resistance to a large number of antibiotics [50]. In recent years, the emergence of methicillin resistance in these bacteria constitutes a real global problem [51]. Indeed, it has been estimated that a 20% mortality rate is associated with infections caused by methicillin-resistant *S. aureus* (MRSA) [52]. MRSA were reported for the first time in 1961 [53]. Their resistance is due to the presence of the penicillin binding protein PBP2a, which has a low affinity to β-lactams. This protein is encoded by the *mecA* gene, which is carried in a mobile genomic element called staphylococcal cassette chromosome *mec* (SCC*mec*) [54].

Worldwide spread of venous catheter infections associated to *Staphylococcus* spp.

Venous catheters are increasingly used and are considered an essential element in modern medicine [55]. It has been reported that more than 80% of hospitalized patients have a central or peripheral intravascular catheter during their stay in hospital [56]. Indeed, a large number of microorganisms have been implicated in medical device associated infections such as intravenous catheters. *S. aureus* and *S. epidermidis* are the most incriminated species [57]. Their ability to form biofilm makes these infections more complicated [15]. These biofilms can be formed in the catheter lumen or on its outer surface [7] and can cause increase in morbidity, mortality, length of hospital stay and hospitalization costs [58]. In Italy, a retrospective study related to infections associated with central venous catheters was conducted over a period of 4 years (from 2007 to 2010). The prevalence of *S. epidermidis* and *S. aureus* was 12.78% and 10.55% respectively during this period [59]. A recent study in Pakistan showed that 64% of catheters were colonized by Gram positive bacteria and *S. aureus* was the most isolated species (39%) followed by *S. epidermidis* (16%) with the prevalence (59%) of MRSA [60]. MRSA infections have been a huge problem in hospitals since their emergence in the 1980s [61]. Khalil and Azqul [62] reported that CNS strains were the most isolates in catheters and 9.9% of patients hospitalized in cardiac care unit had a catheter related-bloodstream infection. Other recent studies conducted in Sweden, Iran, Belgium, India, USA and Poland reported also that *Staphylococcus* spp. strains were the most isolated from intravascular catheter infections [21,63-67]. In Algeria, a study [68] at the hemodialysis department of the Setif University Hospital recorded a rate of 22.4% Central Venous Catheter Related-Infections (CVC-RI). Among the causative microoganisms, CNS and *S. aureus* were found at a rate of 23.5% each and were all resistant to methicillin and other antibiotics [68]. These authors also mentioned that the weak hand hygiene and disinfection of the

skin before placement of catheters were the cause of 58.8% and 88.8% of CVC-RI respectively. Furthermore, in Mexico, Pérez-Zárate *et al*. [55] demonstrated that hand washing, use of sterile gown and preparation of medicines were considered as risk factors for biofilm formation and catheter related infections. In China, it was found that 57 out of 1523 hospitalized patients, in a kidney intensive care unit, had central venous Catheter Related Blood Stream Infection (CRBSI) and *S. aureus* was incriminated in 10 cases [69]. MRSA was reported the cause of 40% of hemodialysis catheter-related bacteremia in Taipei Veterans General Hospital-Taiwan [70]. Duration of catheterization was reported as major risk factor for biofilm formation and subsequent bacterial dissemination. Indeed, Passerini *et al*. [71] showed that 81% of venous catheters placed in patients for 1 to 14 days were colonized by bacteria in biofilm. In Mexico, another group of researchers found that patients using a longer central venous catheter were more likely to develop a catheter-related infection [56,68] confirmed also that longer duration of catheterization (≥ 10 days) is considered as risk factor of CVC-RI.

Treatment strategies for *Staphylococcus* spp. biofilm related infections

The infections caused by *Staphylococcus* spp. biofilms constitute a major problem of global public health because of the persistence and recalcitrance of these and the therapeutic failure that biofilms confer on them, increasing the mortality rate, morbidity but also hospital costs. For this, the development of anti-biofilms treatment has become a major priority for researchers for their prevention and eradication [46,72]. Several global studies have described several strategies to prevent and treat these biofilms such as nanoparticles, antimicrobial peptides, enzymes, bacteriophages, ultrasound therapy, photodynamic therapy, antimicrobial catheter lock solution, plants extracts and chelating agents [46].

Antimicrobial peptides: Antimicrobial peptides are positively charged cationic molecules, amphipathic and composed of 11 to 50 amino acids [73,74]. The first peptide discovered was LL-37 cathelicidin of human origin [75]. These molecules have a broad spectrum of activity (Gram negative and positive bacteria, fungi). They act by permeabilization of the cell membranes which leads to the appearance of pores thus leading to bacterial lysis [76]. Recent studies reported the anti-biofilm effect of these peptides. For example, Zapotoczna *et al*. [77] recorded that the peptide D-Bac8c^{2,5Leu} alone or combined with antibiotics seem to be a potential agent to treat *S. aureus* intravenous catheter related infections [78]. Demonstrated the capacity of the peptide Brevinin-1GHa (skin secretion of the frog *Hylarana guentheri*) to destabilize the cell membrane and eliminate the biofilm of *S. aureus*. Shurko *et al*. [79] showed also the strong inhibition of *S. aureus* biofilm by two peptides LL-13 and LL-17, which derivates from LL-37. Recently, a Dutch study showed that the combination of teicoplanin with SAAP-148 or SAAP-276 peptides showed a strong interaction with *S. aureus* biofilms [80]. Another Dutch study reported that SAAP-148 peptide seems to have an excellent antibacterial and anti-biofilm activities against MRSA biofilms and persisters cells [81]. The peptide derivatives (SPLUNC1 Δα4 SPLUNC1 Δα4M1) showed significantly higher anti-biofilm activity than the SPLUNC1 parent molecule and this is due to the α4 fraction, which has this activity. The latter can be improved by increasing the cationic and Tryptophan content (improvement of hydrophobicity and amphipathicity) [82]. Other peptides showed an anti-biofilm activity against *Staphylococcus* spp. as 17BIPHE2 (derivates from the human peptide LL-37) [83],citropin 1.1 (isolated from the frog *Litoria citropa*) and temporin A (isolated from the frog *Rana temporaria*) [84], peptide FLIP7 (isolated from maggots *Calliphora vicina*) [85], KR-12 and KE-18 [86], tachyplesin I (isolated from horseshoe crab) [87].

Nanoparticles: In recent years, it has emerged that nanotechnology is a promising way to treat infections. This technology uses metal nanoparticles that allow the elimination of bacteria by interacting with their components such as proteins, nucleic acids, peptides. This interaction causes the release of reactive oxygen, hydrogen peroxide (H_2O_2), hydroxyl radical (*OH) that disrupt cell membranes leading to bacterial lysis[88]. Moreover, these nanoparticles are characterized by their small size and high surface-to-mass ratio facilitating interactions [46,89]. Demonstrated that rhamnolipids (RL) coated with silver (Ag) and iron oxide (Fe_3O_4) exhibited important activity against *S. aureus* biofilms. This is due to the release of the reactive oxygen molecules. In addition, these RLs modify the hydrophobicity of the surfaces reducing bacterial adhesion [90]. Showed that the synergistic

combination of silver and antibiotics (fosfomycin, daptomycin, vancomycin, oxacillin) has eradicated *S. aureus* biofilm. [88] demonstrated recently the promising effect of Ag-thymol (ATNPs), Ag-usnic acid (AUNPs), Cu-thymol (CTNPs), and Cu-usnic acid (CUNPs) on MRSA biofilms. Other studies demonstrated also the anti-biofilm activity of Nitric Oxide "NO" [91], Zink oxide "ZnO" [92], gold [93] and SiO_2-Gentamicin nanohybrids[94].

Enzymes: Bacterial enzymes allow the dispersion of biofilms by acting on several components of the matrix (proteins, exopolysaccharids, eDNA). The extraction and purification of these enzymes can be used as a new alternative for the prevention and eradication of biofilms. Several groups of enzymes have been studied [37]. The first group is Proteases. In 2013, a serine protease V8 was reported as a promising enzyme to disturb staphylococcal biofilm [95]. Elchinger *et al.* [96] reported also that lavourzyme protease had a significant activity against *S. epidermidis* biofilm.The enzymes lysostaphin, α-amylase, bromelain, and papain tested against *S. aureus* biofilms (cultured in 10% human plasma) showed, after 2 and 24 hours of treatment, a decrease in biofilm biomass: up to 76% for lysostaphin, 97% for α-amylase and 98% for bromelain and papain. The three last proteases seem to be promising agents for biofilm treatment [97]. Similarly, it was reported that the proteinase K (2 µg/ml) inhibited the biofilm of bap-positive *S. aureus* V329, but it showed any effect on their planktonic cells [6]. Deoxyribonucleases can also be used as anti-biofilm agents. The nuclease NucB, isolated from *Bacillus licheniformis* was effective against staphylococcal biofilms [98]. DNase I allowed the disturbance of *S. aureus* biofilm by degradation of eDNA [99]. Another group of enzymes have also been used as a means of dispersing biofilms: glycoside hydrolases. The same study cited previously showed that the Dispersin B disrupted *S. aureus* biofilms by degrading poly (1,6) -N-acetyl-D-glucosamine polysaccharide [99]. Flemming *et al.* [100] showed also the dispersion of *S. aureus* biofilm by adding α-amylase (cleaves glycosidic link α (1,4) and cellulase (cleaves glycosidic link β(1,4). The enzyme that cleaves hyaluronic acid of the animal matrix, hyaluronidase is also able to disrupt aggregates forming biofilms [101]. The effect of Poly-N-Acetylglucosamine (PNAG) depolymerase (DA7) against staphylococcal biofilms have been recently demonstrated by [102]. This hydrolase was able to destabilize the biofilm by destruction of PNAG, the major polysaccharide of the matrix.

Antimicrobial catheter lock solutions: The antimicrobial catheter lock is considered as a novel strategy to treat intravascular catheter infection [103]. Antiseptics such as ethanol and Taurolock have been reported to be effective at eradicating biofilms aged three days or more [104]. The antimicrobial agents ML: 8 (1% v/v) and Citrox (1% v/v), which are generally used in the treatment of periodontal infections, were able to reduce staphylococcal biofilm biomass (> 97%) after only 24 hours of treatment [105]. Recent Spanish study showed that the administration of the combination of ethanol (40%) and heparin (60 IU) during 72 hours was effective *in vitro* against staphylococcal biofilm [106].Fibrinolytic agents as plasmin, streptokinase, and nattokinase (alone or combined with antibiotics) were successfully efficient in disturbance of *S. aureus* biofilms in rat model of intravascular catheter infection *in vivo* [103]. Bhatt *et al.* [107] demonstrated the anti-biofilm effect of a novel antibiotic-free formed from gas Plasma-Activated Disinfectant (PAD). This agent allowed reducing the biomass of the staphylococcal biofilm (MRSA and *S. epidermidis*) after 1 hour and a re-growth of the bacteria was not observed after 24 hours of incubation, making it a promising means for the eradication of biofilms. Other studies reported also the antimicrobial catheter lock solutions [108-111].

Antibodies: Since the production of new antibiotic molecules has become limited in recent years, researchers have resumed their interest in treating monoclonal antibodies as an alternative for preventing biofilm and treating infections that directly targets the pathogen [47,112]. An American study showed that PhnD-specific antibodies (Phosphonate ABC transporter substrate binding protein) inhibited *S. aureus* biofilm formation by preventing their fixation and aggregation [113]. Den Reijer *et al.* [114] showed that the immunogenic IsdA and SA0688 seem to be potential novel agents to prevent or treat *Staphylococcus* biofilm-associated infections. The *in vivo* effect of human antibody TRL1068 alone or with vancomycin against MRSA biofilms was investigated using a model of catheter-induced aortic valve infective endocarditis in rats. The results showed significant reductions in biofilm [112].

Bacteriophages: Bacteriophages are natural viruses that infect and replicate inside host bacteria, causing cell lysis and the release of new virions that infect other target bacteria. As a result, phages can enter biofilms; regulate the cell growth, making them a promising strategy for treating bacterial infections [115-116]. These phages have more advantages unlike antibiotics such as their activity on multi-drug resistant strains, low harmful effect on eukaryotic host cells, rapid reproduction and ease of isolation and low cost of production but mainly their high anti-biofilm potential [116]. In 2017, an American study investigated the anti-biofilm potential of phage lysin CF-301 against staphylococcal biofilms. The authors showed that this phage-lysin disrupted biofilm with minimal biofilm eradicating concentration 90 (MBEC90) values of 0.25 µg/ml (MRSA). CF-301 had an anti-biofilm activity at very low concentrations against MRSA biofilms formed on catheters, but also on mixed biofilms (*S. aureus* and *S. epidermidis*) [117]. Tkhilaishvili *et al*. [118] showed that bacteriophage Sb-1 had a synergistic effect with specific antibiotics ((fosfomycin, rifampin, vancomycin, daptomycin or ciprofloxacin) for the eradication of MRSA strains by degrading their extracellular matrix. The synergistic effect of the combination of *PYO* phage and antibiotics was also studied by Dickey and Perrot [119]. Other studies have also reported the potential anti-biofilm effect of phages [102,115,120,121].

Plants extracts: Since antiquity, humans use plants and their extracts as drugs for the treatment of infections [122]. In fact, these plants had several anti-inflammatory, antibacterial, antithrombotic, anti-allergic, hepato-protective and anti-carcinogenic activities [46]. The plant components that have strong antibacterial activity are classified as phytoalexins[123]. In addition, essential oils are also considered effective alternatives to antibiotics and antiseptics [124]. These aromatic oils are extracted from aromatic plants and contain various secondary molecules that play a protective role for plants [125]. In recent years, several studies have been interested in the anti-biofilm activities of plant extracts. For example, in 2016, an Iranian study demonstrated *in vitro* a strong anti-biofilm activity of coriander essential oil (*Coriandrum sativum L.*) against *S. aureus* with a Minimal Inhibitory Concentration (MIC) of 0.8 µl /ml [122]. Gandhi *et al*. [123] studied the effect of *Sesbania grandiflora* extracts against *S. aureus* biofilm and observed that this plant, which contains several molecules (alkaloids, flavonoids, saponins and tannins), inhibited carbohydrates and proteins (major components of the *S. aureus* biofilm matrix) thereby reducing biofilm formation. Khan *et al*. [126]. Found that the ethanolic extract of *Zanthoxylum armatum* fruits inhibited the quorum sensing of *S. aureus* (IC50= 32–256 µg/ml). Another recent Chinese study showed that peppermint essential oil inhibited *S. aureus* biofilm formation at 0.25 mg/ml and eradicated it at concentration ≥4 mg/ml [124]. Other studies have reported the anti-biofilm activity of *Aloe vera* [127], *Allium stipitatum* [128], *Syzygium cumini* L. Skeels [129] extracts, and *Lippia sidoides*, *Thymus vulgaris* and *Pimenta pseudochariophyllus* essential oils [130].

Metal chelators, sulfhydryl compounds: Another group of molecules with antimicrobial activity is the chelating agents that act on the biofilm by sequestering metal ions (iron, magnesium and calcium). These metal ions play an important role in the production of many proteins involved in biofilm formation such as adhesins and proteases [131]. Maisetta *et al*. [132] demonstrated that Ethylenediamine Tetra-Acetic Acid (EDTA) combined with the peptide (temporin 1Tb) was able to kill *S. epidermidis* in biofilm on silicone catheters . Martinez-Andrade *et al*. [133] showed during their *in-vitro* study that 17% EDTA combined with silver nanoparticles (AgNPs) had antimicrobial activity against *S. aureus* biofilms. In addition, this cationic chelator destabilizes biofilms and prevents their adhesion [134]. An Australian study reported that the iron chelator "deferiprone" seems to be a promising molecule to treat *Staphylococcus* biofilms by disrupting the synthesis of the PIA [72]. This iron chelator in combination with antibiotics eradicated the *S. epidermidis* biofilm [135]. Another study recorded also the anti-biofilm effect of iron chelators 2,2′-dipyridyl (2DP) and 1,2,3,4,6-Penta-O-Galloyl-b-D-Glucopyranose (PGG) on *S. aureus* biofilm formation [136]. On the other hand, other molecules such as sulfhydryl compounds such as dithiothreitol, beta-mercaptoethanol and cysteine also appear to have a significant antibacterial effect against *S. aureus* biofilm. These molecules are able to inhibit the formation of PIA, major component of the matrix of Staphylococci [46,72]. The study cited previously demonstrated that the use of a sulfhydryl compound (L-cycteine) at a concentration of

78µM in combination with the peptide 1Tb had reduced the biosynthesis of *S. epidermidis* ATCC 35984 PIA[132].

Nitric oxide: Nitric oxide (NO) is a diatomic and lipophilic gaseous molecule that plays a role in cellular signaling and immunity. This molecule has also an anti-inflammatory, bactericidal and bacteriostatic effect [137]. Furthermore, the NO signaling molecule plays a major role in dispersion of biofilms, which has been associated with a conditional reduction of c-di-GMP leading to matrix degradation and bacterial motility [138]. Several recent studies reported its positive effect on *Staphylococcus* biofilm making it a good alternative in therapeutic [137,139,140].

Photodynamic, Ultrasound and Laser Shock waves therapy: Photodynamic therapy is a new antibacterial alternative using photosensitizing molecules activated in the presence of O_2 by the light generating an oxidation of the biological molecules causing the destruction of the target microorganism. The advantage of this therapy is the lack of selection of resistance, but also, the low cost of treatment and the low risk of side effects [141]. Several studies reported that the photosensitizer 5-aminolevulinicacid (alone or combined with antibiotics) [142], $ZnPc^{n+}$ [143], Chlorin e6 (Ce6) [144], RB (RB-aPDT) (alone or combined with gentamicin) [141] and toluidine blue O [145] seem to be potential alternatives to treat *S. aureus* biofilm related infections. Other therapies such as ultrasound and laser shock waves seem also to give good results in the eradication of biofilms. The advantage with these physical methods is that they are non-invasive, do not cost much and the risk of inducing bacterial resistance is very low [146-148].

CONCLUSION

This review allowed us to point out that the emergence of catheter-related infections caused by staphylococcal biofilm, particularly SARM, and linked to high mortality and morbidity, is a universal public health problem. Surveillance of these infections has become a challenge for clinicians and microbiologists. Measures and training on hygiene rules and placement of venous catheters should be taken to control the onset of these infections and in particular to ensure patient safety. In addition, the major problem with these infections is that the biofilm confers on bacterial populations increased resistance to antibiotics causing a therapeutic failure. As a result, the development of new strategies as an alternative to antibiotics has become a real challenge for the control and eradication of staphylococcal biofilms in recent years. Despite the discovery of many strategies, unfortunately, some of them cannot be administered to patients such as chelators, nanoparticles toxic at certain doses and bacteriophages. Further research should be based on natural molecules such as antimicrobial peptides, which look promising for the treatment of infections. In addition, several researchers have so far demonstrated the *in vitro* anti-biofilm potential of most molecules and therapies (alone or in combination). However, additional studies need to be conducted to validate these findings, such as cytotoxicity, genotoxicity and pharmacology of the molecules, as well as *in vivo* studies.

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Novel Antibacterial Agents SAAP-148 and Halicin Combat Gram-Negative Bacteria Colonizing Catheters

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Abstract: The antibiotic management of catheter-related infections (CRIs) often fails owing to the emergence of antimicrobial-resistant strains and/or biofilm/persister apparitions. Thus, we investigated the efficacy of two novel antimicrobial agents, i.e., the synthetic peptide SAAP-148 and the novel antibiotic halicin, against Gram-negative bacteria (GNB) colonizing catheters. The antibacterial, anti-biofilm, and anti-persister activities of both agents were evaluated against *Acinetobacter baumannii*, *Escherichia coli*, and *Klebsiella pneumoniae* strains. The enrolled strains were isolated from catheters and selected based on their resistance to at least three antibiotic classes and biofilm formation potential. Furthermore, the hemolysis and endotoxin neutralization abilities of these agents were explored. The bactericidal activity of both agents was reduced in urine and plasma as compared to buffered saline. In a dose-dependent manner, SAAP-148 and halicin reduced bacterial counts in 24 h preformed biofilms on silicone elastomer discs and eliminated persisters originating from antibiotic-exposed mature 7-day biofilms, with halicin being less effective than SAAP-148. Importantly, SAAP-148 and halicin acted synergistically on *E. coli* and *K. pneumoniae* biofilms but not on *A. baumannii* biofilms. The peptide, but not halicin, decreased the production of IL-12p40 upon exposure to UV-killed bacteria. This preliminary study showed that SAAP-148 and halicin alone/in combination are promising candidates to fight GNB colonizing catheters.

Keywords: catheter; antibiotic resistance; biofilm; persisters; halicin; SAAP-148

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1. Introduction

Catheters are essential in the management of a range of clinical scenarios, such as the delivery of chemotherapy, antibiotics, and parenteral nutrition, as well as (hemo)dialysis [1,2]. Unfortunately, their use is complicated by their propensity to become colonized by bacteria, which may lead to serious infections [3,4], such as bloodstream and recurrent urinary tract infections, that affect significantly the length of hospital stays, mortality rates, and costs [5]. Catheter-related bloodstream infections (CRIs) are some of the most frequent, lethal, and costly complications of central venous catheterization [6], with incidences ranging from 1.8 to 5.2 per 1000 catheters [7]. In addition, urinary catheterization accounts for 40% of all the hospital-acquired infections in the United States [8] and possibly worldwide [9]. For many years, Gram-positive staphylococci were reported as the most common causative agents of CRIs, followed by Gram-negative bacteria (GNBs) [4,10–12]. However, in recent years, the epidemiology and microbiology of CRIs have changed, and a shift in the predominance of CRI pathogens from Gram-positive to GNBs, such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter* spp., is widely reported [13–17]. These bacteria may originate from the

endogenous flora of the patients (e.g., from mucosa) or from exogenous sources, such as other patients, healthcare workers, (hospital) environmental surfaces, or contaminated objects [18]. The treatment of patients with an infected catheter often involves the removal of the infected device, followed by intensive antibiotic therapy [19]. The failure of this treatment is caused by the emergence of antimicrobial-resistant strains [20,21] and/or the formation of biofilms [11,22] and persisters [23]. Upon the elimination of the stress, persisters revive and can start a new infection, which explains the persistence of these CRIs. Moreover, biofilms are considered as major contributors to persistent infections, constituting a global health problem [24]. Because of these considerations, novel antibacterial and anti-biofilm/persister agents for the treatment of CRIs are urgently required. Antimicrobial peptides (AMPs), which are molecules of the first line of defense against infections [25], are considered as promising candidates as they combine broad-spectrum antimicrobial activities with immune-modulating capabilities [26–30]. Interestingly, these peptides kill bacteria by mechanisms different from those of current antibiotics, such as interacting with and subsequently disrupting the microbial plasma membrane. Moreover, some AMPs can interact with the extracellular polysaccharides of the matrix, leading to the disintegration of biofilms [31,32]. One of the best-studied AMPs is human cathelicidin LL-37 [32–35], from which the LL-37-based synthetic antimicrobial and anti-biofilm peptide (SAAP)-148 was developed using random amino acid substitutions in the C-terminal part of LL-37 [28]. In a study by de Breij et al. [28], the peptide SAAP-148 was highly efficient in killing a panel of planktonic multidrug-resistant (MDR) bacteria, including colistin-resistant *E. coli*, *K. pneumoniae*, and *A. baumannii*. Further studies have also shown the efficacy of this peptide against biofilms [28,36] and persister cells [28,37,38]. de Breij et al. [28] investigated the mode of action of SAAP-148 and showed that the formation of pores in cell walls led to the permeabilization of the membrane, followed by the destruction of the bacterial cells. Recently, using computer-assisted deep learning discovery approaches (at the Drug Repurposing Hub) [39], halicin was identified as the first-in-class novel small molecule based on the probability of being able to inhibit *E. coli* growth. Importantly, additional assays demonstrated that halicin exhibits broad-spectrum antibacterial activities against clinical strains, such as carbapenem-resistant *Enterobacteriaceae*, MDR *A. baumannii* and *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, and *Clostridium difficile*, in vitro and in a murine infection model [39]. Likewise, this agent was demonstrated as being effective against MDR *A. baumannii* 3086 [40] and 24 h immature and 7-day-mature biofilms formed on a polypropylene plate [36]. Moreover, it was suggested that the compound kills bacteria by dissipating the transmembrane electrochemical gradient, ΔpH, after binding with iron [39,41]. The effect of either SAAP148 or halicin on Gram-positive bacteria, especially *S. aureus*, has been extensively studied [28,36–38,40,42–50]. However, few reports on their effects on GNBs are available [28,36,39,40,46–49,51], and none of the studies tested strains isolated directly from medical devices and using both agents against GNBs. In this preliminary study, we investigated the antibacterial efficiencies of SAAP-148 and halicin as potential candidates for the development of a novel treatment for CRIs associated with GNBs. This study is the first one exploring in the same work the anti-biofilm and anti-persister activities of SAAP-148 and halicin alone and in combination against GNB strains isolated from catheters.

2. Results

2.1. Identification of the Bacterial Strains Colonizing Catheters

A total of 40 GNB strains isolated from 10 intravenous catheters (IVCs) and 4 urinary catheters (URCs) were identified using 16S rDNA sequencing. *Enterobacteriaceae*, predominantly *E. coli*, were the most abundant (81%) in the IVCs; the remainder (19%) was *Acinetobacter* spp., predominantly *A. baumannii* (Table 1). Similarly, all the bacterial strains isolated from the URCs were *Enterobacteriaceae*, e.g., *K. pneumoniae* (56%) and *E. coli* (44%) (Table 1).

Table 1. Characteristics of Gram-negative bacterial strains isolated from intravenous and urinary catheters.

Identity	Origin	* Resistance Profile	** Biofilm Mass (OD Values)	*** Classification [52]	**** Virulence Profile
Klebsiella pneumoniae KP1	URC	AMC^R; FEP^R; TET^R; GEN^R	0.292 ± 0.041 ↓↓	Strongly adherent	*mrkD+; fimH+; ycfM+; ecpA+*
Klebsiella pneumoniae KP2	URC	AMC^R; CTX^R; CAZ^R; FEP^R; TET^R; GEN^I	0.254 ± 0.022 ↓↓	Strongly adherent	*mrkD+ fimH+; ycfM+; ecpA+*
Klebsiella pneumoniae KP3	URC	AMC^R; CTX^R; CAZ^R; FEP^R; TET^R; GEN^R	0.121 ± 0.031 ↓↓	Strongly adherent	*mrkD+; fimH+; ycfM+; ecpA+*
Klebsiella pneumoniae KP4	URC	AMC^R; CTX^R; CAZ^R; FEP^R; TET^R	0.187 ± 0.011 ↓↓	Strongly adherent	*mrkD+; fimH+; ycfM+; ecpA+*
Klebsiella pneumoniae KP5	URC	AMC^R; CTX^R; CAZ^R; FEP^R; TET^R; CIP^I; GEN^I	0.074 ± 0.031 ↓	Moderately adherent	*mrkD+; fimH+; ycfM+; ecpA+*
Acinetobacter baumannii AB1	IVC	CTX^R; CAZ^R; FEP^R; IPM^R; TET^R; CIP^R; CO-TRI^R	0.242 ± 0.020 ↓↓	Strongly adherent	*csuE+; ompA+; bap+*
Acinetobacter baumannii AB2	IVC	CTX^R; CAZ^R; FEP^R; IPM^R; TET^R; CIP^R; CO-TRI^R	0.088 ± 0.013 ↓↓	Moderately adherent	*csuE+; ompA+; bap+*
Acinetobacter baumannii AB3	IVC	CTX^R; CAZ^R; FEP^R; IPM^R; TET^R; CIP^R; CO-TRI^R	0.057 ± 0.025 ↓	Weakly adherent	*csuE+; ompA+; bap+*
Acinetobacter lwoffii AL1	IVC	FEP^R; IPM^R; TET^R; CIP^R; CO-TRI^R	0.349 ± 0.048 ↓↓	Strongly adherent	*csuE−; ompA+; bap−*
Acinetobacter baumannii AB4	IVC	CTX^R; CAZ^R; FEP^R; IPM^R; TET^R; CIP^R; CO-TRI^R	0.059 ± 0.159 ↓	Weakly adherent	*csuE+; ompA+; bap−*
Acinetobacter baumannii AB5	IVC	CTX^R; CAZ^R; FEP^R; IPM^R; TET^R; CIP^R; CO-TRI^R	0.021 ± 0.013	Non-adherent	*csuE+; ompA+; bap+*
Escherichia coli EC1	IVC	TET^R; NA^R	0.03 ± 0.013	Non-adherent	*fimH+; hlyF+; csgA+*
Escherichia coli EC2	URC	TET^R; GEN^I; NA^R	0.376 ± 0.022 ↓↓	Strongly adherent	*fimH+; hlyF−; csgA+*
Escherichia coli EC3	IVC	*Sensitive to all antibiotics*	0.028 ± 0.01	Non-adherent	*fimH+; hlyF−; csgA+*
Escherichia coli EC4	URC	NA^R	0.101 ± 0.019 ↓↓	Moderately adherent	*fimH+; hlyF−; csgA+*
Escherichia coli EC5	URC	CIP^I; NA^R	0.259 ± 0.007 ↓↓	Strongly adherent	*fimH+; hlyF+; csgA+*
Escherichia coli EC6	IVC	NA^R	0.261 ± 0.032 ↓↓	Strongly adherent	*fimH+; hlyF+; csgA+*
Escherichia coli EC7	IVC	NA^R	0249 ± 0.032 ↓↓	Strongly adherent	*fimH+; hlyF−; csgA+*
Escherichia coli EC8	IVC	TET^I; NA^R	0.292 ± 0.024 ↓↓	Strongly adherent	*fimH+; hlyF+; csgA+*
Escherichia coli EC9	IVC	TET^R; NA^R	0.222 ± 0.012 ↓↓	Strongly adherent	*fimH+; hlyF+; csgA+*
Escherichia coli EC10	IVC	AMC^R; NA^R	0.284 ± 0.018 ↓↓	Strongly adherent	*fimH+; hlyF-; csgA+*
Escherichia coli EC11	IVC	AMC^R; CO-TRI^R	0.027 ± 0.011	Non-adherent	*fimH+; hlyF−; csgA+*
Escherichia coli EC12	URC	NA^R	0.068 ± 0.022 ↓	Moderately adherent	*fimH+; hlyF−; csgA+*
Escherichia coli EC13	IVC	CIP^I	0.243 ± 0.015 ↓↓	Strongly adherent	*fimH+; hlyF−; csgA+*
Escherichia coli EC14	IVC	AMC^R; CO-TRI^R	0.051 ± 0.007 ↓	Weakly adherent	*fimH+; hlyF−; csgA+*
Escherichia coli EC15	IVC	AMC^R; TET^R	0.044 ± 0.019 ns	Weakly adherent	*fimH+; hlyF+; csgA+*
Escherichia coli EC16	IVC	TET^R; NA^R	0.094 ± 0.008 ↓↓	Moderately adherent	*fimH+; hlyF−; csgA+*
Escherichia coli EC17	IVC	TET^R; NA^R	0.141 ± 0.017 ↓↓	Strongly adherent	*fimH+; hlyF−; csgA+*
Escherichia coli EC18	IVC	CIP^I; NA^R	0.096 ± 0.011 ↓↓	Moderately adherent	*fimH+; hlyF+; csgA+*
Escherichia coli EC19	IVC	TET^R; NA^R	0.047 ± 0.034 ns	Weakly adherent	*fimH+; hlyF−; csgA+*
Escherichia coli EC20	IVC	CIP^I; NA^R	0.131 ± 0.032 ↓↓	Strongly adherent	*fimH+; hlyF+; csgA+*
Escherichia coli EC21	IVC	TET^R; NA^R	0.04 ± 0.023 ns	Weakly adherent	*fimH+; hlyF+; csgA+*
Escherichia coli EC22	IVC	AMC^R; NA^R	0.122 ± 0.003 ↓↓	Strongly adherent	*fimH+; hlyF+; csgA+*
Escherichia coli EC23	IVC	CIP^I; NA^R	0.105 ± 0.007 ↓↓	Moderately adherent	*fimH+; hlyF+; csgA+*
Escherichia coli EC24	IVC	TET^R; NA^R	0.101 ± 0.029 ↓↓	Moderately adherent	*fimH+; hlyF+; csgA+*
Escherichia coli EC25	IVC	TET^R; NA^R	0.042 ± 0.015 ns	Weakly adherent	*fimH+; hlyF+; csgA+*
Escherichia coli EC26	IVC	AMC^R; NA^R	0.039 ± 0.006 ns	Weakly adherent	*fimH+; hlyF+; csgA+*
Escherichia coli EC27	IVC	TET^R; NA^R	0.024 ± 0.003	Non-adherent	*fimH+; hlyF+; csgA+*
Escherichia coli EC28	IVC	AMC^R; CO-TRI^R	0.023 ± 0.011	Non-adherent	*fimH+; hlyF−; csgA+*
Escherichia coli EC29	IVC	*Sensitive to all antibiotics*	0.052 ± 0.008 ↓	Weakly adherent	*fimH+; hlyF−; csgA−*

IVC: intravenous catheter; URC: urinary catheter; bacteria were coded "EC" for *Escherichia coli*, "AB" for *Acinetobacter baumannii*, and "KP" for *Klebsiella pneumoniae*. *Only intermediate (I)/resistance(R) is indicated. Tested antibiotics: AMC: amoxicillin + clavulanic acid; CTX: cefotaxime; CAZ: ceftazidime; FOX: cefoxitin; ERY: erythromycin; TET: tetracycline; CIP: ciprofloxacin; GEN: gentamycin; IMP: imipenem; FEP: cefepime; NA: nalidixic acid; CO-TRI: cotrimoxazole. ** Biofilm mass; n = 3 independent experiments each in triplicate. Mann–Whitney U test: ↓ $p < 0.05$ indicates that OD_{sample} is significantly different from $OD_{control}$; ns: not significantly different. $OD_{control} = 0.03$. *** The strains were classified as follows: non-adherent ($OD_{sample} \leq 0.03$); weakly adherent ($0.03 < OD_{sample} \leq 0.06$); moderately adherent ($0.06 < OD_{sample} \leq 0.12$); strongly adherent ($OD_{sample} > 0.12$). **** Virulence genes; +: gene present; −: gene absent.

2.2. Bacterial Resistance to Antibiotics

Virtually all the *E. coli* strains were sensitive to almost all the tested antibiotics, except for nalidixic acid (76%), tetracycline (34%), amoxicillin/clavulanic acid (24%), and cotrimoxazole (10%), while the *K. pneumoniae* isolates were resistant to β-lactams (except for cefoxitin), and two out of the five strains were resistant to gentamicin. The *Acinetobacter* isolates exhibited high levels of resistance to all the antibiotics, except for gentamicin (Table 1).

2.3. Bacterial Biofilm Formation and Virulence Gene Profiles

To further characterize these catheter-colonizing strains, their ability to form biofilms and the presence of genes involved in adherence to surfaces and biofilm formation were assessed. The results revealed that biofilm formation on polystyrene microplates varied widely among the *E. coli* (only 11/29 were strong formers) and *Acinetobacter* (only 2/6 were strong formers) strains, while all the *K. pneumoniae* strains formed significant biofilms (Table 1); 4/5 were strong producers. In addition, all 29 *E. coli* strains harbored the *fimH* gene; 28/29 strains, the *csgA* gene; and 15 out of the 29 strains (≈52%), the *hlyF* gene. All six *K. pneumoniae* strains harbored the *mrkD*, *fimH-1*, *ycfM*, and *ecpA* genes. In addition, all five *A. baumannii* strains exhibited the *ompA* and *csuE* genes, whereas four out of the five strains exhibited the *bap* gene (Table 1).

2.4. Bactericidal Efficacies of SAAP-148 and Halicin on Selected Strains

Based on the above characterization data, four GNBs (*E. coli* EC2, *A. baumannii* AB1, and *K. pneumoniae* KP1 and KP2), resistant to at least three antibiotics, were selected for further studies on the antibacterial properties of SAAP-148 and halicin. The results revealed that in a dose-dependent manner, SAAP-148 in PBS killed all four bacterial strains, with LC 99.9% values at concentrations ranging from sub-micromolar to low micromolar (Table 2). In addition, in a dose-dependent manner, the peptide was effective against these bacteria in 50% (*v*/*v*) pooled human urine and 50% (*v*/*v*) human plasma, although 4–8× higher peptide concentrations were required. Similar bactericidal activities were obtained after 4 and 24 h of exposure to the peptide. Also, in a dose-dependent manner, halicin killed all four bacterial strains in PBS, with LC 99.9% values ranging between 6.4 µM and 51.2 µM depending on the strain (Table 2). Halicin was more effective after 24 h than after 4 h of exposure. Of note, halicin was less effective against KP1, KP2, and EC2 in pooled urine than in PBS and virtually ineffective against AB1 in pooled plasma (Table 2). These results revealed that the peptide SAAP-148 and halicin are potent candidates as antibacterial drugs against GNBs.

Table 2. Bactericidal efficacies of SAAP-148 peptide and halicin against antibiotic-resistant Gram-negative strains isolated from catheters.

Strains	Antibiotic Resistances											LC 99.9% of SAAP-148 (µM)				LC 99.9% of Halicin (µM)			
												4 h		24 h		4 h		24 h	
	AMC	CTX	CAZ	FEP	FOX	IPM	TET	CIP	GEN	NA	CO-TRI	PBS	50% Plasma or Urine	PBS	50% Plasma or Urine	PBS	50% Plasma or Urine	PBS	50% Plasma or Urine
E. coli EC2												0.8	3.2	0.8	12.8	25.6 (12.8–25.6)	102.4 (51.2–102.4)	6.4	25.6 (12.8–25.6)
A. baumannii AB1										/		0.8	1.6	0.8	3.2	25.6	>102.4	25.6	>102.4
K. pneumoniae KP1												0.8 (0.8–1.6)	6.4	1.6	12.8	51.2 (25.6–51.2)	25.6	51.2	51.2
K. pneumoniae KP2												1.6 (0.8–1.6)	6.4 (3.2–6.4)	1.6	12.8	25.6 (12.8–25.6)	51.2 (25.6–51.2)	25.6	102.4 (51.2–102.4)

An in vitro killing assay was used to determine the bactericidal activities of SAAP-148 and halicin when administered in PBS; 50% urine (EC2, KP1, and KP2); and 50% human plasma (AB1). Approximately 2% (*v*/*v*) of the bacteria were mixed with increasing concentrations of SAAP-148 or halicin and after 4 h and 24 h, the number of surviving bacteria was assessed microbiologically. The results are expressed as LC 99.9% values, i.e., the lowest concentration of the antimicrobial agent that results in a 99.9% reduction in the bacterial count compared to that of the control. The values are the means (and ranges) of six replicates from three independent experiments. If no range is mentioned, the LC 99.9% was identical in all the experiments. AMC: amoxicillin + clavulanic acid; CTX: cefotaxime; CAZ: ceftazidime; FEP: cefepime; FOX: cefoxitin; IMP: imipenem; TET: tetracycline; CIP: ciprofloxacin; GEN: gentamycin; NA: nalidixic acid; CO-TRI: cotrimoxazole; sensitive: green box; resistant/intermediate: red box; not tested: white box.

2.5. Reduction in Bacterial Counts in Biofilms by SAAP-148 and Halicin

Because biofilms are central to CRIs, we next determined the abilities of SAAP-148 and halicin to eradicate 24 h biofilms on a silicone elastomer, a surface mimicking a catheter. After 4 h of exposure, the BBC99.9 values for the peptide against EC2, AB1, KP1, and KP2 significantly amounted to 51.2 µM, 25.6 µM, 102.4 µM, and 102.4 µM, respectively; while after 24 h of exposure, they were higher (>102.4 µM) (Figure 1). Identically, halicin was effective against the four-strain biofilm (BBC99.9 ≈ 102.4 µM). The agent was highly

effective at a low concentration (~25.6 μM) against the EC2 biofilm compared with the other strains, which required high concentrations (~204.8 μM). These data indicated that the compounds could be promising anti-biofilm agents.

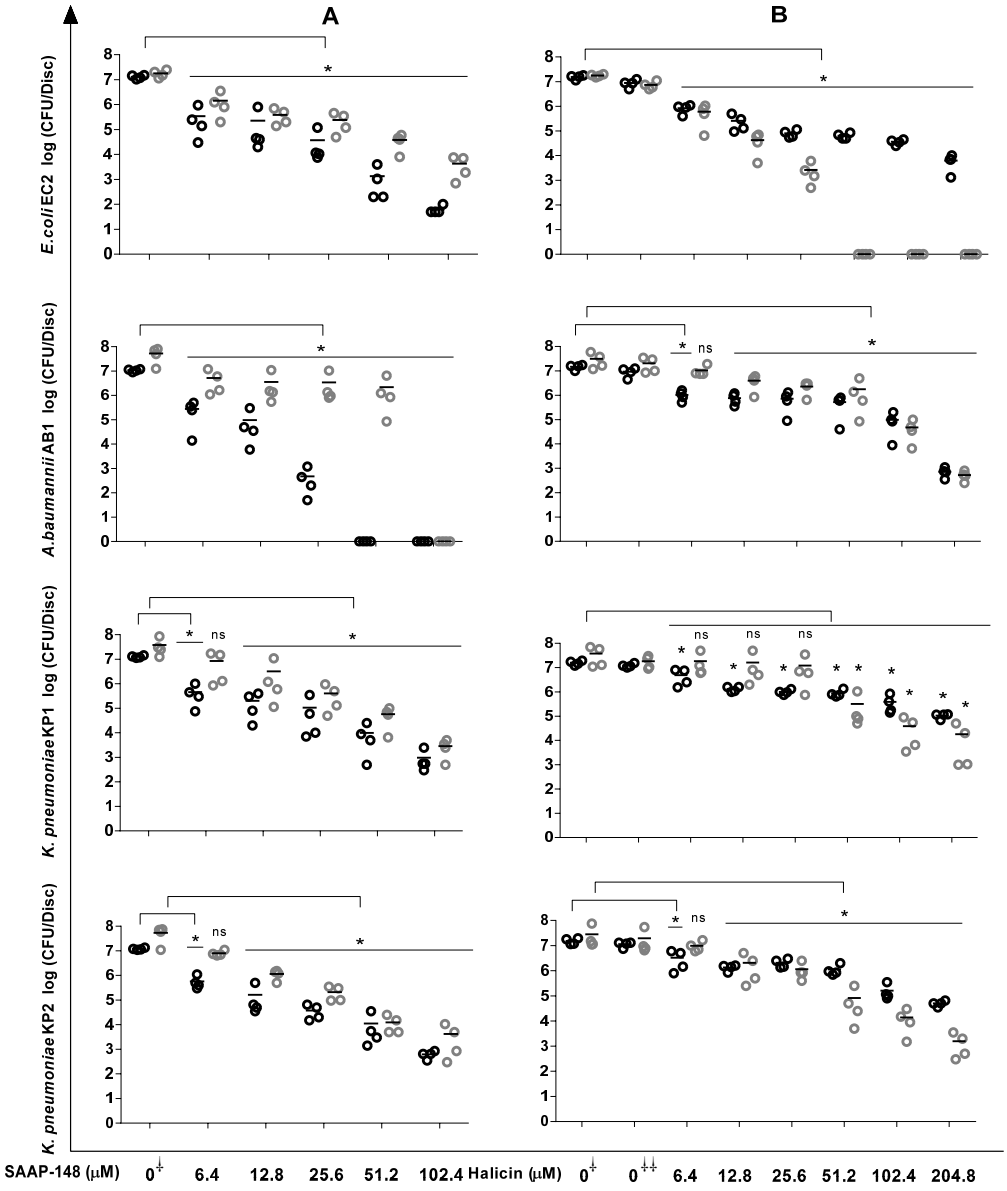


Figure 1. Reduction in bacterial counts within biofilms upon exposure to SAAP148 and halicin. In short, 24 h Gram-negative biofilms on silicone elastomer discs were exposed for 4 h and 24 h to increasing concentrations of SAAP-148 (**A**) and halicin (**B**); then, the biofilms were sonicated, and the number of surviving bacteria was assessed microbiologically. The results are expressed as the mean of the number of viable bacteria in $\log_{10}$ CFU/disc. Four experiments were undertaken in triplicate. The data obtained after 4 h and 24 h exposures are represented by open black and open grey symbols, respectively. Mann–Whitney U test: * $p < 0.05$ indicates significantly different from control (0↓, no agent). DMSO control (0↓↓): control for halicin, i.e., the concentration of DMSO in the highest concentration of halicin that was tested. ns: not significantly different. Kruskal–Wallis test indicated that SAAP-148 as well as halicin at 4 h and 24 h were significantly effective against all the strains.

2.6. Effects of SAAP-148 and Halicin on Persisters Derived from Antibiotic-Exposed Mature Biofilms

Because persisters play a pivotal role in chronic infections and antibiotic treatment failure, we assessed the effects of SAAP-148 and halicin on persisters derived from 7-day mature biofilms that had been exposed to high doses of antibiotics for an additional three days. As expected, high doses (50 × MBC) of ciprofloxacin significantly ($p < 0.01$) reduced ($\geq 100\times$) the numbers of viable bacteria in the EC2, KP1, and KP2 biofilms, as did similarly high doses of gentamycin for the bacterial counts in the AB1 ($p < 0.01$) biofilms, without the complete elimination of all the bacteria (Figure 2). However, SAAP-148 was highly effective in killing persisters derived from antibiotic-exposed mature EC2, AB1, KP1, and KP2 biofilms (Figure 2), with the complete eradication of the EC2 and AB1 persisters at 1.6 μM. For KP1 and KP2, slightly higher concentrations, i.e., 3.2 μM and 6.4 μM of the peptide were required, respectively (Figure 2). Interestingly, the EC2 and AB1 persisters were eliminated by high concentrations of halicin, whereas the KP1 and KP2 persisters were not eradicated by this agent (Figure 2).

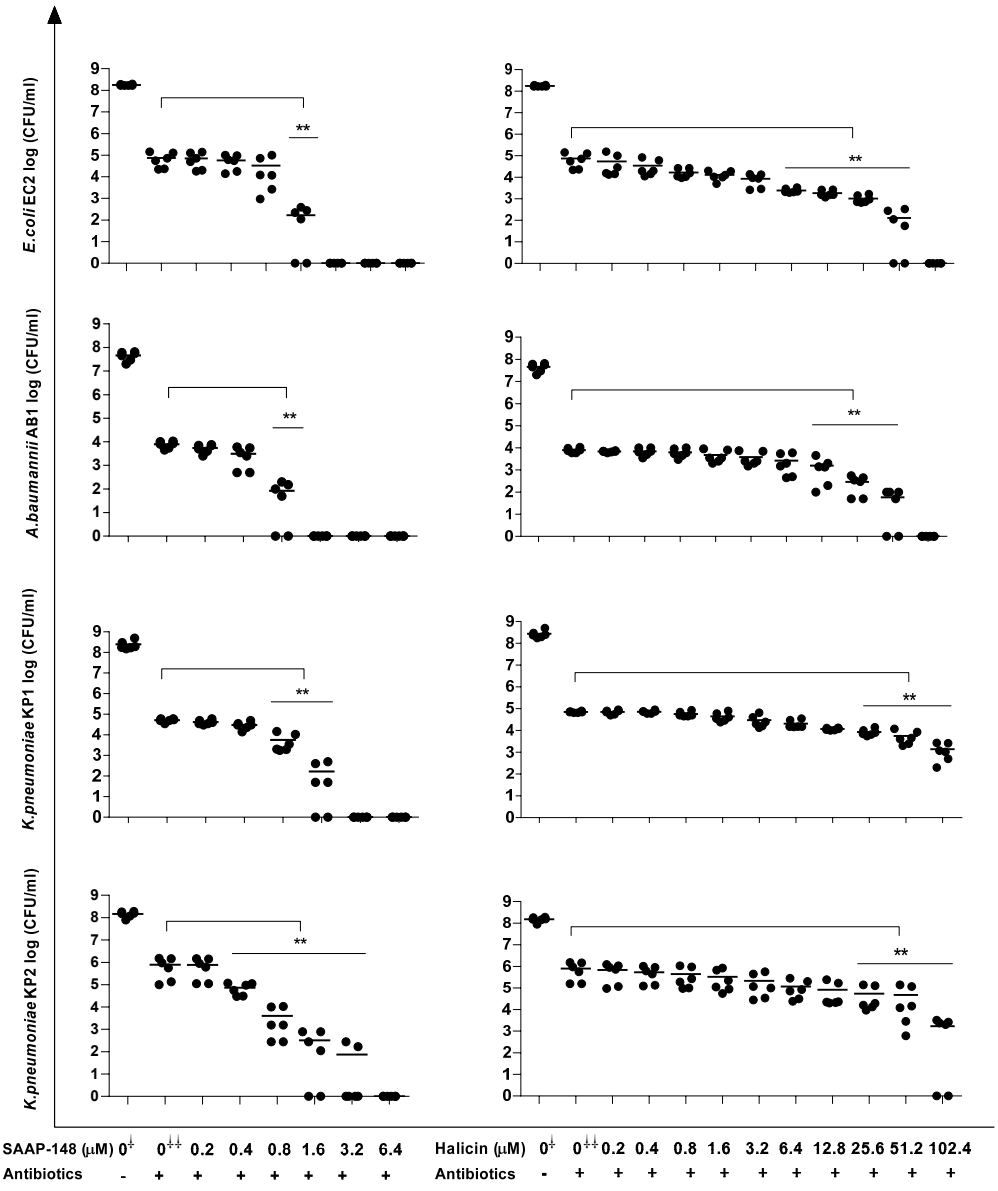


Figure 2. Effects of SAAP-148 and halicin on persisters derived from antibiotic-exposed mature biofilms. In short, bacterial biofilms were cultured for seven days, washed, exposed for 3 days to

antibiotics, washed, and then exposed for 4 h to increasing concentrations of the peptide or halicin. Thereafter, the biofilms were sonicated to obtain a suspension of persisters, enabling the microbiological detection of the bacterial counts. The results are expressed as the mean of the number of viable bacteria in $\log_{10}$ CFU/mL. Three independent experiments in duplicate were undertaken. To enrich for persisters, *E. coli* EC2 and *K. pneumoniae* KP1 and KP2 were exposed to 50 × MBC ciprofloxacin; *A. baumannii* AB1, 50 × MBC gentamicin. The Kruskal–Wallis test indicated that SAAP-148 and halicin were significantly effective against all the persisters at 4 h. $0_{\downarrow}$: number of bacteria before treatment with antibiotic. $0_{\downarrow\downarrow}$: number of bacteria after 3 days of exposure to the antibiotic. ** $p < 0.01$ (Mann–Whitney U test) indicated significant differences from controls exposed to the antibiotic ($0_{\downarrow\downarrow}$) but not SAAP-148/halicin.

2.7. SAAP-148 and Halicin Neutralize GNB-Induced IL-12p40 Production by Human Blood Leukocytes

We compared the abilities of SAAP-148 and halicin to reduce the capacities of EC2, AB1, KP1, and KP2 to stimulate the production of the pro-inflammatory cytokine IL-12p40 by human blood leukocytes. The results revealed that in a concentration-dependent manner, the pre-incubation of UV-inactivated bacteria with SAAP-148, but not halicin, reduced the ability of the strains to stimulate IL-12p40 production by blood leukocytes (Figure 3).The concentrations of SAAP-148 that significantly ($p < 0.05$) reduced the abilities of UV-inactivated EC2, AB1, KP1, and KP2 to induce cytokine production amounted to ≥1 nM, ≥10 nM, ≥10 nM, and ≥10 nM, respectively (Figure 3). These preliminary data revealed that the synthetic peptide exhibited promising activity as an anti-inflammatory agent.

2.8. Hemolytic Activities of SAAP-148 and Halicin

To obtain some insight into the in vitro toxicities of SAAP-148 and halicin, we assessed the hemolytic activities of these compounds using human erythrocytes suspended in 50% (***v/v***) human plasma and in PBS. The highest concentrations of SAAP-148 that lysed ≤5% of the erythrocytes in the PBS and 50% plasma amounted to <12.8 μM and 51.2 μM (Figure 4A,B), respectively. The hemolytic activity of the halicin amounted to ≥204.8 μM in the PBS and 50% plasma (Figure 4C,D, respectively), indicating that halicin is less hemolytic than the peptide.

2.9. Interactions between SAAP-148 and Halicin

As the modes of action underlying the antibacterial activities of SAAP-148 and halicin differ [28,39], the possibility of synergistic and/or additive interactions between these novel agents in eradicating bacteria in biofilms was investigated using checkerboard assays. The results revealed that several combinations of SAAP-148 and halicin were more effective than each agent alone in reducing bacterial counts in EC2, KP1, and KP2 biofilms but not in AB1 biofilms (Table 3). Based on the fractional biofilm eradication concentration index (ΣFBEC), some combinations of SAAP-148 and halicin acted synergistically on bacteria in EC2, KP1, and KP2 but not AB1 biofilms, whereas other combinations exerted additive effects (Table 3). Importantly, the concentrations of SAAP-148 needed to eliminate the bacteria in biofilms in the presence of halicin were considerably lower than that required for the peptide alone.

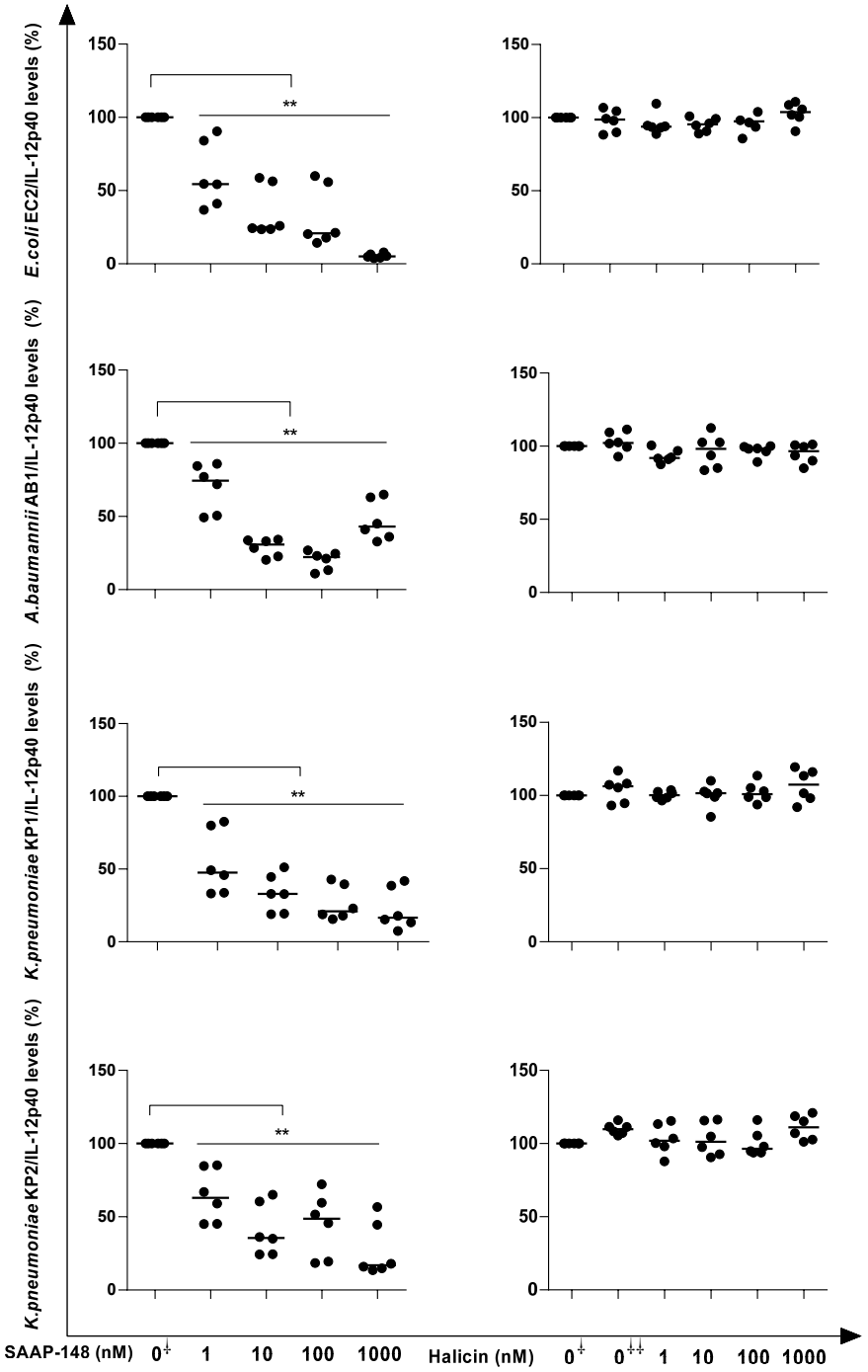


Figure 3. Pre-incubation for 2 h of UV-inactivated GNB strains with SAAP-148, but not halicin, reduced the ability of GNBs to induce IL-12-p40 production by whole human blood leukocytes. In short, UV-killed bacteria were pre-incubated with increasing concentrations of SAAP-148 or halicin (from 1 to 1000 nM) for 2 h and then mixed with 5-fold diluted whole blood. After 20 h of incubation at 37 °C, the cells were spun down, and the levels of IL-12p40 in the supernatants were assessed using ELISA. The results are expressed as the percentage of IL-12p40 compared with that of the control (0↓), i.e., UV-inactivated bacteria exposed to PBS instead of the peptide. 0↓↓: UV-inactivated bacteria exposed to the highest concentration of DMSO instead of the halicin concentration that was tested. Values are the means of three independent experiments, each performed in duplicate. The Kruskal–Wallis test indicated that SAAP-148 significantly reduced the abilities of all the bacteria (EC2 and AB1: $p < 0.0001$; KP1: $p = 0.0004$ and KP2: $p = 0.0005$) to induce IL-12p40 production. ** $p < 0.01$ compared to the control (no peptide) using Mann–Whitney U test.

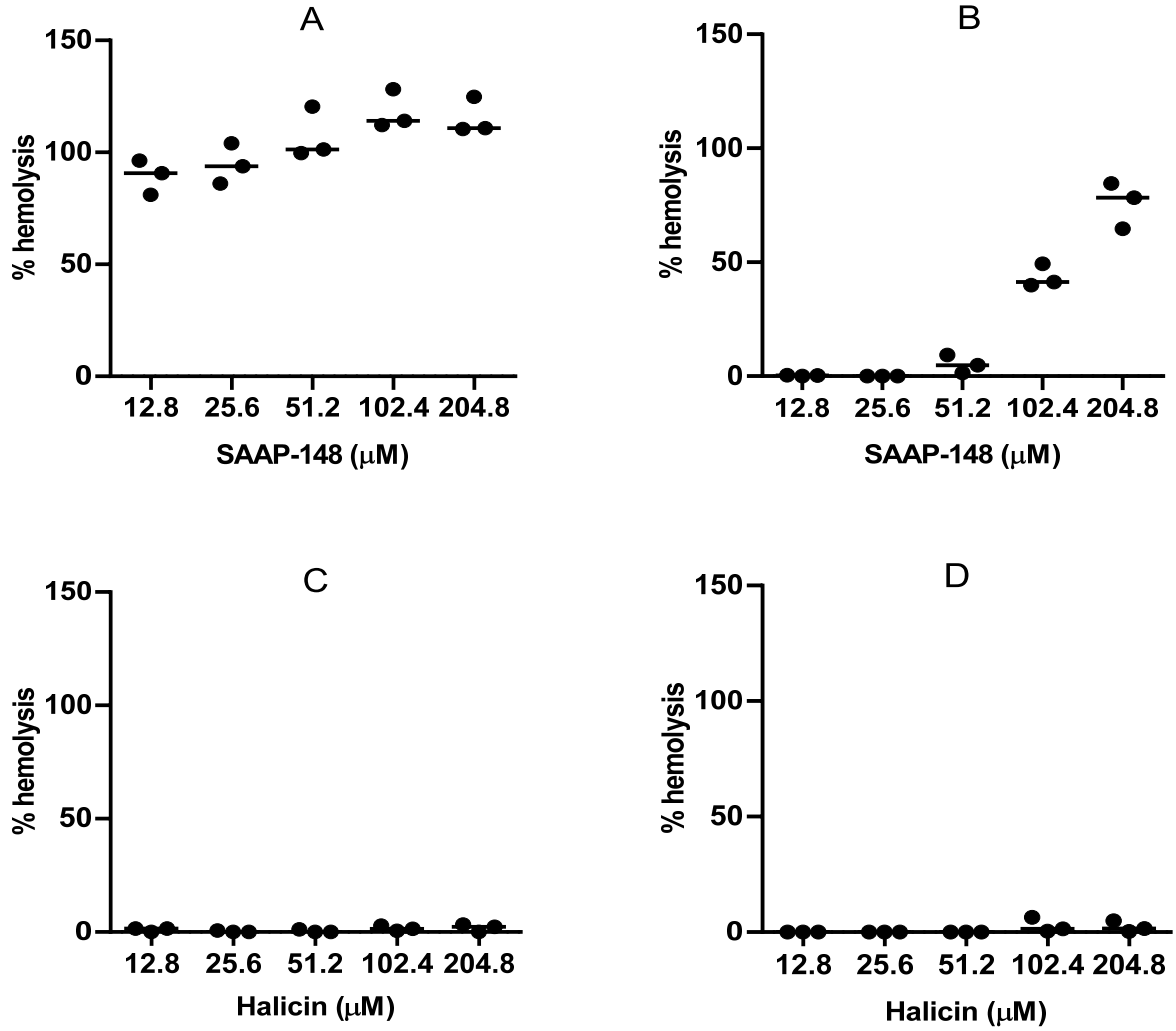


Figure 4. Hemolytic activities of SAAP-148 in PBS (**A**) and 50% plasma (**B**) and of halicin in PBS (**C**) and 50% plasma (**D**). Values are means of three independent experiments, each performed in triplicate.

Table 3. Synergistic effects of SAAP-148 and halicin alone/in combination against GNB preformed biofilms and fractional biofilm eradication concentration indexes (ΣFBEC) for combinations of SAAP-148 and halicin.

Strain	MBEC SAAP-148 (μM) Alone	MBEC SAAP-148 (μM) in Combination	MBEC Halicin (μM) Alone	MBEC Halicin (μM) in Combination	ΣFBEC
E. coli EC2	102.4	3.2	25.6	6.4	0.28 (Synergistic effect)
		3.2		12.8	0.53 (Additive effect)
		6.4		12.8	0.56 (Additive effect)
		12.8		6.4	0.38 (Synergistic effect)
		12.8		12.8	0.63 (Additive effect)
		25.6		6.4	0.5 (Synergistic effect)
		25.6		12.8	0.75 (Additive effect)
A. baumannii AB1	102.4	No effect	102.4	No effect	No effect
K. pneumoniae KP1	51.2	12.8	102.4	12.8	0.38 (Synergistic effect)
		12.8		25.6	0.5 (Synergistic effect)

Table 3. *Cont.*

Strain	MBEC SAAP-148 (μM) Alone	MBEC SAAP-148 (μM) in Combination	MBEC Halicin (μM) Alone	MBEC Halicin (μM) in Combination	ΣFBEC
K. pneumoniae KP2	51.2	3.2	102.4	102.4	1.06 (Indifferent effect)
		12.8		12.8	0.38 (Synergistic effect)
		12.8		25.6	0.5 (Synergistic effect)
		12.8		51.2	0.75 (Additive effect)
		12.8		102.4	1.25 (Indifferent effect)
		25.6		51.2	1 (Additive effect)
		25.6		102.4	1.5 (Indifferent effect)

In short, 24 h biofilms on sterile silicone elastomer discs were washed twice and then exposed to increasing concentrations of SAAP-148 and halicin (alone) and to different concentrations of SAAP-148 and halicin (in combination) for 24 h at 37 °C. Thereafter, the discs were washed twice after the gentle removal of the antimicrobial agents, and the remaining biofilms were sonicated for 10 min at 42 kHz. The number of surviving bacteria was determined microbiologically. The experiment was assessed four times each in duplicate. MBEC: minimum biofilm eradication concentration. Synergy is defined by the fractional biofilm eradication concentration index (ΣFBEC) $\leq$ 0.5; additive effect as 0.5 < ΣFBEC $\leq$ 1; indifference as 1 < ΣFBEC $\leq$ 2; antagonism as ΣFBEC > 2.

3. Discussion

The present study is the first undertaken in Bejaia (in the northeast of Algeria) and, thus, constitutes a precious source of information for the region and the country. As recently reported, a predominance of GNBs (40 out of 50 catheter-colonizing strains) was observed rather than Gram-positive ones, which explains our focus on these bacteria in this study. Furthermore, according to Buetti et al. [53], most epidemiological studies so far have neglected to focus on GNBs as causes of catheter infections. *E. coli* (~81%) and *A. baumannii* (16%) were the predominant species in the intravenous catheters, and this could be explained by the local prevalence of these ubiquitous pathogens in the hospital environment [54–58]. It is well documented that the hospital environment (e.g., bed rails, mattresses, medical equipment, colonized or infected patients, and the hands of healthcare workers) is an ecological niche for *A. baumannii* and *E. coli* [59–61]. For urinary catheters, *K. pneumoniae* was the most isolated species in this study (~56%), followed by *E. coli* (44%), which is consistent with the results found by Barbadoro et al. [62] but not by others [21,63–65]. In Algeria, the predominance of *E. coli* in urinary tract infections is widely reported [66–68], but no epidemiological data are available for catheter-colonizing uropathogens. It is not surprising to find these two uropathogens because *E. coli* and *K. pneumoniae* are fecal contaminants or residents of patients' native or transient microflora that colonize the peri-urethral area [69].

Another observation pertains to the relation between the biofilm formation and presence of genes related to this process. As all the bacteria were derived from colonized catheters, we expected 100% of the strains to form a biofilm on polystyrene surfaces. However, if this was true for *K. pneumoniae* isolates (100%), only 83% (24/29) of the *E. coli* and 83.3% (5/6) of the *Acinetobacter* isolates were biofilm formers, which is in agreement with other reports [70–72]. Of course, it cannot be excluded that the characteristics of the surface play a role in biofilm formation. Therefore, in further experiments, we used silicone elastomers, which more accurately mimic medical devices, such as venous and urinary catheters [73]. Biofilm formation is mediated by multiple virulence factors, such as fimbriae (*fimH*) and curli (*csgA*), for *E. coli* [74]; CsuE, a subunit of the chaperone–usher pili (Csu), the outer membrane protein (OmpA), and the biofilm-associated protein (Bap), for *A. baumannii* [75–77]; and fimbriae type 1 (*fimH-1*) and type 3 (*mrkD*), for *K. pneumoniae* [78]. In the present study, these genes were present in all the strains. However, a comparison between the biofilm-forming and non-biofilm-forming isolates revealed that the presence of the virulence factors was not associated with biofilm production.

Surprisingly, the *E. coli* strains were sensitive to almost all the antibiotics that were tested. These results are not in agreement with other Algerian studies reporting a prevalence of high resistance to β-lactams, gentamicin, and ciprofloxacin, except for imipenem, which was active against *E. coli* strains [67,79–81]. Our results showed that a high rate of biofilm-

forming *E. coli* strains (83%) was more sensitive to antibiotics, indicating no clear correlation between the ability to form a biofilm and sensitivity to antibiotics. This can be explained by the fact that the strains form biofilms to survive when exposed to antibiotics [82]. Another explanation could be that the polymeric matrix acts as a barrier and protects the bacteria from antibiotics, thereby preventing the penetration of the biofilm by the antibiotics. In addition, the binding of antibiotics to matrix components may reduce the activities of the antibiotics [83–85]. Alves et al. [86] reported that 73% of positive biofilm-forming *E. coli* isolates were sensitive to antibiotics. Poursina et al. [87] also concluded that non-MDR *E. coli* strains were able to form strong biofilms. The sensitivity of the strains to imipenem could be explained by its rare use in Algerian hospitals. We found that the *K. pneumoniae* strains harbored high rates of resistance to amoxicillin/clavulanic acid and ß-lactams (expect for cefoxitin), tetracycline, and gentamycin, while all the strains were sensitive to imipenem, nalidixic acid, ciprofloxacin (except for the KP5 strain), and cotrimoxazole. The majority of the Algerian studies used GNB clinical isolates, but a few Algerian studies enrolled catheter-colonizing strains. High resistance to antibiotics has also been reported in other countries [78,88–91]. The *Acinetobacter* spp. strains exhibited high resistances to ß-lactams (expect for cefoxitin), imipenem, tetracycline, ciprofloxacin, and cotrimoxazole, but no strain was resistant to gentamycin, which is in agreement with antibiotic resistances in *A. baumannii* strains reported by other Algerian researchers [55,80,92,93].

It must be noted that the main objective of this study was the evaluation of the antibacterial and anti-biofilm effects of SAAP-148 and halicin alone or in combination on GNB-colonizing catheters. We used single strains of *E. coli* and *A. baumannii* and two *K. pneumoniae* strains. The results of the killing assay revealed that SAAP-148 and halicin are highly active against the tested strains. This observation is in agreement with earlier reports showing the effectiveness of SAAP-148 [28] and halicin [39] against a wide range of bacteria, including MDR GNBs (*E. coli*, *A. baumannii*, *P. aeruginosa*, and *K. pneumoniae*). Contrariwise, the activities of both agents were reduced in human plasma and urine. As already reported, the antimicrobial activities of peptides and antibiotics could decrease in the presence of these physiological fluids owing to the presence of components preventing the interaction of these agents with bacterial membranes [94,95]. In addition, SAAP-148 and halicin significantly reduced the bacterial counts within biofilms, and the required concentrations were considerably higher than those required for directly killing planktonic cells. This can be attributed to the biofilm matrix, which acts as a barrier that delays or prevents the interaction of antimicrobial agents with bacterial cells, thereby reducing the effectiveness of the agents against the biofilms [96]. The results obtained with SAAP-148 are consistent with previous studies showing its efficacy against GNB biofilms [28,36]. Notably, compared with the study by van Gent et al. [36], where a high concentration (68.3 μM) of halicin was needed, this study revealed that halicin displayed the strongest effect on the *E. coli* biofilm at a low concentration (~25 μM). These findings suggest that the compounds have a promising anti-biofilm activity.

The presence of persister cells, which can survive in up to high doses of antibiotics, is mainly responsible for chronic infections and their recalcitrance [97]. In the present work, we exposed mature biofilms to high concentrations (50 × MBC) of ciprofloxacin (EC2, KP1, and KP2) and gentamycin (AC1) for 3 days. These antibiotics induced a deep disruption of the matrix, leading to their penetration of the biofilms [98,99] and, thus, significantly reducing the number of bacteria within them. It has been suggested that the decrease in the activities of these antibiotics is correlated with persister cells [82]. SAAP-148 and halicin were effective against these persisters derived from the antibiotic-exposed mature biofilms of the four bacterial strains tested in a dose-dependent manner. This is in agreement with earlier findings that SAAP-148 effectively reduced bacterial counts within antibiotic-exposed mature biofilms of MRSA [37,38]. Herein, we provided proof for the first time that SAAP-148 possesses a potent anti-persister effect against GNB strains. Further, it should be kept in mind that planktonic, sessile, and persister bacteria all play important

roles in CRIs, including catheter-related bloodstream infections and recurrent urinary tract infections [100,101].

The development of molecules able to kill GNBs without releasing their endotoxins (LPS), which are located in the outer membrane and induce severe pro-inflammatory responses, is a major challenge [102,103]. Peptides are able to bind to LPS and act as anti-inflammatory agents against endotoxic shock [104,105]. In our study, we found that SAAP-148, but not halicin, was able to reduce the ability of UV-killed GNBs to induce IL-12-p40 cytokine production in vitro. Furthermore, these results are reported for the first time, indicating that this synthetic peptide could be an excellent candidate as an anti-inflammatory agent (in vitro) to treat sepsis caused by GNB infections. In vivo experiments to monitor organ injury in mouse models of LPS-induced endotoxemia and *GNB*-induced septic shock could give more evidence about the efficiency of SAAP-148, as reported in research by Jang et al. [102]. Several hypotheses on the mechanism of action of peptides that can induce an anti-inflammatory response have been reported. First, cationic peptides bind to LPS via electrostatic interactions, thereby neutralizing anionic amphiphilic lipid A. Second, the peptide binds to the macrophage's CD14 receptor and competitively inhibits the interaction of the LPS–LPB complex and, thus, blocks access to the TLR4 receptor, which mediates pro-inflammatory cytokines. Third, most LPS-binding peptides are able to dissociate LPS oligomers by depolymerization, thereby inhibiting LPS from binding to LBP [106,107].

In addition to antibacterial, anti-biofilm, and anti-inflammatory activities, low toxicity against human red blood cells (RBCs) is an important parameter for novel agents to be used as potential candidates in the treatment of biofilm-related infections [108]. We demonstrated that compared with halicin, which has no effect on RBCs, the peptide SAAP-148 is more toxic toward erythrocytes. The cytotoxicity assay, which is not included herein but is reported in a study by van Gent et al. [36], also showed that SAAP-148 was more cytotoxic than halicin for skin fibroblasts and RT-4 urethelial cells. It is known that hydrophobicity is crucial for peptides to disintegrate bacterial membranes. It has been reported that peptides possessing high hydrophobicity are correlated with high toxicity [36,109]. The increasing hydrophobic content (tryptophan, lysine, and arginine) leads to a significantly high hemolytic activity [110,111]. Moreover, SAAP-148 possesses a larger hydrophobic region in its amino-acid sequence [112]. The ability to form amphipathic structures has also been related to increased hemolytic activity [113]. Using SAAP-148 in combination with other agents could reduce the concentration of the peptide, thereby reducing the peptide's toxicity. Interestingly, recent studies have reported improved strategies for designing potent and safe peptides for therapeutics, such as PLGA (poly(lactic-co-glycolic acid)) nanoparticles [49] and C-terminal PEGylation [47], which seem to be promising approaches to overcome these drawbacks.

Furthermore, synergistic and additive interactions were noted between SAAP-148 and halicin against *E. coli* and *K. pneumoniae*, but not *A. baumannii*, in biofilms. These findings are in agreement with previous observations that the peptide and halicin act synergistically against planktonic MRSA but not against MDR *A. baumannii* [36]. Unfortunately, we cannot give an explanation for these species/strain-specific interactions between SAAP-148 and halicin. However, other researchers have reported similar findings for SAAP-148 and classical antibiotics [42] and for other antimicrobial peptides combined with other antimicrobial agents against GNB biofilms [114–118]. Importantly, synergistic combinations of the peptide and halicin enable the use of lower doses of SAAP-148, possessing good antibacterial activities, while limiting harmful side effects in vivo. The use of antimicrobial peptides in combination with conventional/novel antibiotics or other antimicrobial agents could be an effective strategy to reduce the development of resistance [119]. Future medical applications of (combinations of) SAAP-148 and halicin can be both invasive and non-invasive. Of note, combination treatment faces several hurdles, including the cytotoxicity and poor PK/PD properties of SAAP-148. However, conjugates of SAAP-148 and halicin could overcome several limitations encountered by the combinations. Regarding non-invasive usage,

(combinations or conjugations of) SAAP-148 and halicin may be considered for antibiotic lock therapy [120,121], where (i) the catheter can be coated with the agents encapsulated in a polymeric formulation, such as hydrogel-loaded peptides and peptide-releasing hydrogels [122–124], or (ii) the agents may be immobilized on the surface of the catheter [122]. The use of antimicrobial agents as a coating is the most popular approach owing to their ability to target microorganisms in different ways [125]. Several studies have reported the effectiveness of the use of antibiotics [126–128] and antimicrobial peptides [129,130] as coatings for eradicating/preventing the colonization of pathogens on catheter surfaces. Alternatively, the agents may be used invasively, e.g., immediately after the removal of a colonized catheter or as a treatment for patients with a CRI in whom the current treatment has failed.

It should be mentioned that the current in vitro study has some limitations. First, all the experiments were performed on a single strain of *E. coli* and *A. baumannii* and two *K. pneumoniae* strains from one hospital. Another limitation of this study is that the experiments were performed under static conditions (microtiter plate assays), whereas colonized catheters in situ are constantly subjected to a dynamic flow of blood or urine. This limitation may be circumvented in future experiments using flow cells, e.g., a modified Robbin's device, in which a biofilm is formed in an environment of plasma or urine flowing under a constant shear (reviewed by Subramanian et al. [131]).

4. Materials and Methods

4.1. Isolation of Bacteria from Catheters

Between February and April 2016, 14 catheters (4 urinary and 10 intravenous catheters) were removed from 14 patients (5 women and 9 men) hospitalized for at least 48 h in the departments of resuscitation, general surgery, and internal medicine at Bejaia University Hospital (Bejaia, Algeria) because of infectious disease complications. The age of the patients ranged from 27 to 96 years, and the duration that the catheter was *in situ* ranged from 2 to 16 days. The bacteria were harvested from the catheters using the method of Brun Buisson et al. [132] with minor modifications. For this purpose, the tip of the catheter was cut off, transferred to 10 mL of a sterile tryptone salt solution (TS; 0.1% w/v), and then sonicated for 5 min at 42 kHz (ultrasonic cleaner, Bransonic, Saint Louis, MO, USA). The bacterial suspension was vortexed for 1 min, after which the bacteria were spread on nutrient agar (NA, Conda, Madrid, Spain) and trypticase soy agar (TSA, Biokar Diagnostics, Allonne, France) plates for the enumeration of the total flora and on eosin methylene blue agar (EMB, Conda, Madrid, Spain) plates to assess the *Enterobacteriaceae* counts. The remaining catheters were washed and resuspended in 10 mL of nutrient broth (NB, Conda, Madrid, Spain). After incubation at 37 °C for 24–48 h, streak isolations were prepared from each positive broth on the specific agar plates mentioned above. The bacteria were identified using conventional methods.

4.2. Identification of the Strains

All the Gram-negative isolates were genotypically identified using 16S rDNA sequencing. Briefly, single colonies were obtained from the NA plate using a sterile plastic disposable loop, and bacterial DNA was extracted using 20 µL of lysis buffer (0.25% (w/v) SDS, 0.05 N NaOH) and by heating for 15 min at 95 °C. The DNA samples were rapidly cooled and centrifuged at 13,000 rpm for a few seconds (Beun De Ronde, Abcoude, the Netherlands). Next, 180 µL of milli-Q water was added, and the DNA solution was mixed thoroughly, centrifuged again for 5 min at 13,000 rpm, and stored at −20 °C until use. PCR was used to amplify approximately 1500 bp of a consensus 16S rDNA gene: forward primer αβ NOT (5′-AGT TTG ATG CTG GCT CAG-3′) and reverse primer ω MB (5′-TAC CTT GTT ACG ACT TCG TCC CA -3′). Two microliters of the DNA sample, 2 µL of the 10 µM forward and reverse primers, 25 µL of the master mix (Promega, Madison, WI, USA), and 21 µL of milli-Q water were mixed and subjected to the following program: 1 × 5 min at 95 °C; 3 × 1 min at 95 °C; 2 min at 50 °C; 1 min at 72 °C; 35 × (45 s at 95 °C, 45 s at 50 °C,

and 1 min at 72 °C); and 1 × 5 min at 72 °C. The amplified products were analyzed using electrophoresis (Bio-Rad, Hercules, CA, USA) on 1% (*w*/*v*) agarose (Roche Diagnostics, Mannheim, Germany) gel at 120 V for 2 h. The PCR product was cleaned as follows: 0.5 µL of *ExoI* (20 units/µL; Thermo Scientific, Vilnius, Lithuania) and 1 µL of *Fast AP* (1 unit/µL; Thermo Scientific) were added to 5 µL of the PCR product and incubated at 37 °C for 15 min. The enzymes were then deactivated by heating at 85 °C for 15 min, and the mixture was finally diluted 10× with Milli-Q water. For sequencing, 5 µL of the PCR product, 1 µL of the forward and reverse primers (1 pmol/µL) and 4 µL of Milli-Q water were mixed. The sequencing was performed on an Applied Biosystems 96-capillary system, ABI3730xl with a pop7 matrix (Macrogen, Amsterdam, the Netherlands).

4.3. Antibiotic Susceptibility Testing

The antibiotic susceptibility of the strains was tested using the disc diffusion method [133]. Briefly, bacterial suspensions of 0.5 McFarland were seeded on Mueller–Hinton agar (Becton Dickinson, Mississauga, ON, Canada) plates. The plates were air-dried for 15 min and then the discs impregnated with antibiotics were deposited on the plates (antibiotics in Supplementary Table S1). The diameter of the inhibition zone around each disc was measured after 24 h at 37 °C, and the strains were graded as sensitive (S), intermediate (I), or resistant (R) following the European Committee on Antimicrobial Susceptibility Testing guidelines [134]. *Escherichia coli* ATCC25922 was included as a reference strain for quality control.

4.4. Detection *of Bacterial Virulence and Adhesion Genes*

The presence of virulence and adhesion genes in the clinical isolates was assessed using PCR. Briefly, 50 µL of a buffer containing bacterial DNA; 25 µL of the master mix (Taq polymerase, polymerase buffer (pH 8.5), and 400 µM dNTPs2x (Promega)); and 10 µM forward and reverse primers (specific primers in Supplementary Table S2) were mixed. The PCR programs are indicated in Supplementary Table S3.

4.5. Biofilm Formation on Polystyrene Microplates

The biofilm formation on sterile flat-bottom 96-well polystyrene microplates (Greiner Bio-One, Frickenhausen, Germany) was assessed using the method by O'Toole [135]. The wells of the microplate, previously filled with 100 µL of trypticase soy broth (TSB, Biokar Diagnostics), were seeded in triplicate with 100 µL of an 18 h bacterial culture (1×10^6 CFU/mL) and incubated at 37 °C for 24 h. Wells containing 200 µL of sterile TSB broth were included as negative controls. After incubation, the cultures were removed; the wells were washed carefully with 200 µL of sterile TS solution, and the biofilms were fixed with 200 µL of absolute ethanol (Biochem Chemopharma, Montreal, Quebec, Canada). Subsequently, the fixed biomass was washed, stained with 0.1% (*w*/*v*) crystal violet solution (Biochem Chemopharma) for 30 min, and finally washed with 200 µL of TS solution. The biomass was finally quantified using a microplate reader (Chromate Awareness Technology, Palm City, FL, USA) to measure the absorbance at 630 nm for the crystal violet extracted with 96% (*v*/*v*) ethanol. The results were expressed as mean values.

4.6. Novel Antibacterial Agents

The SAAP-148 peptide (amide-LKRVWKRVFKLLKRYWRQLKKPVR-acetyl; Mw 3970) was synthesized using 9H-fluorenylmethyloxycarbonyl (Fmoc) chemistry in an automated peptide synthesizer (Syro II, MultiSyntech, Witten, Germany), as described previously [28,136]. The molecular mass of the peptide was confirmed using mass spectrometry (Voyager DE-Pro, PerSeptive Biosystems, Framingham, MA, USA), and the purity amounted to >95%, as determined using reverse-phase high-performance liquid chromatography (HPLC) and detection at 214 nm [137]. The lyophilized peptide was stored at −20 °C until use. A stock solution of 5.12 mM SAAP-148 was prepared in Milli-Q water and stored in the refrigerator at 4 °C. Halicin (SU 3327; Mw 261.3) was purchased from Tocris Bioscience (Bristol, UK).

Prior to use in the experiments, the halicin was dissolved in 748 µL of dimethyl sulfoxide (DMSO, Honeywell Riedel-de Haën, Seelze, Germany) and further diluted in phosphate-buffered saline (PBS, 140 mM, pH 7.4; Fresenius KABI, Zeist, the Netherlands) to a stock solution of 51.2 mM.

4.7. In Vitro Killing Assay

From glycerol stock suspensions of *K. pneumoniae* (KP1 and KP2), *A. baumannii* (AB1), and *E. coli* (EC2), overnight cultures were prepared at 37 °C. Thereafter, the bacteria were cultured to the mid-log phase in TSB (Oxoid, Basingstoke, UK) for 2.5 h under continuous rotation, centrifuged at 3000 rpm for 10 min, and resuspended in PBS to a concentration of 5×10^6 CFU/mL. Subsequently, 30 µL of PBS containing SAAP-148 or halicin (final concentrations ranging from 0.8 to 102.4 µM) and 2% (*v*/*v*) bacterial suspension were mixed with pure PBS; PBS supplemented with 1% (*v*/*v*) TSB; or PBS supplemented with 50% (*v*/*v*) pooled human plasma (for AB1) or 50% (*v*/*v*) pooled urine (for KP1, KP2, and EC2) in the wells of a polypropylene V-shape microplate (Greiner bio-one). After incubation for 4 h and 24 h at 37 °C (200 rpm), the number of viable bacteria was assessed microbiologically. The results were expressed as lethal concentration (LC) 99.9, i.e., the lowest concentration of the compound that killed 99.9% of the *inoculum*.

4.8. Anti-Biofilm Assay

One-hundred microliters of a suspension of log-phase bacteria (approximately 10^7 CFU/mL in brain heart infusion (BHI) broth, Oxoid, Basingstoke, UK) were added to each well of a flat-bottom 96-well polystyrene microplate containing a sterile silicone elastomer disc (Ø = 4 mm), which mimics medical devices, like intravenous and urinary catheters, and incubated for 24 h at 37 °C. Thereafter, each disc was gently transferred in a sterile 96-well microplate, washed with PBS, and transferred again in a sterile flat-bottom 96-well polystyrene plate for exposure to increasing concentrations of SAAP-148 (in the range 6.4–102.4 µM) or halicin (in the range 6.4–204.8 µM) for 4 h and 24 h at 37 °C. Three discs were incubated with PBS as controls. In addition, three discs were exposed to 0.4% (*v*/*v*) DMSO. The concentration of DMSO in the highest concentration of halicin that was tested served as a control for halicin. After the treatment, each disc was washed as described above; transferred to a sterile, flat-bottom, 96-well polystyrene microplate containing 100 µL of PBS; and sonicated for 10 min at 42 kHz. The number of surviving bacteria was determined microbiologically. The results were expressed as biofilm bactericidal concentrations (BBC99.9), i.e., the lowest concentrations of the agents resulting in a 99.9% reduction in the bacterial counts in the biofilms.

4.9. Checkerboard Assay for Determination of SAAP-148 and Halicin Synergy

A checkerboard assay was used to determine the possible synergy between SAAP-148 and halicin against bacteria in biofilms. In short, 24 h biofilms formed on silicone elastomer discs, as described above, were washed two times with 100 µL of PBS and then exposed to combinations of increasing doses of SAAP-148 and halicin (Supplementary Table S4) for 24 h at 37 °C. Thereafter, the antimicrobial agents were gently removed; the plates were washed two times with PBS and sonicated for 10 min at 42 kHz. The number of surviving bacteria was determined microbiologically. The fractional biofilm eradication concentration index (ΣFBEC) was calculated according to the following formula: $\Sigma FBEC = FBEC_A + FEBC_B$ [138], where $FBEC_A$ = MBEC of compound A in the combination/MBEC of compound A, and $FBEC_B$ = MBEC of compound B in the combination/MBEC of compound B. Synergy was defined as $\Sigma FBEC \leq 0.5$; additive effect, $0.5 < \Sigma FBEC \leq 1$; indifference, $1 < \Sigma FBEC \leq 2$; antagonism, $\Sigma FBEC > 2$.

4.10. Anti-Persister Assay

Seven-day mature biofilms were developed in flat-bottom, 96-well polypropylene plates using 100 µL of 1×10^7 CFU/mL bacteria in BHI (Oxoid) broth. The plates were

sealed and incubated at 37 °C in a humidified atmosphere for 7 days. Thereafter, non-adherent bacteria were removed by two washes with PBS, and 100 µL of fresh BHI broth containing 50 × MBC of ciprofloxacin (25 µg/mL; Sigma-Aldrich, St. Louis, MO, USA) for KP1, KP2, and EC1 and 50 × MBC of gentamycin (400 µg/mL; Sigma-Aldrich) for AB1 were carefully added to each well. The medium containing the antibiotics was refreshed daily for 3 days. After the treatment, the supernatant was removed; the biofilm was sonicated at 42 kHz for 10 min, and the resulting bacterial suspensions from 48 wells were pooled. A total volume of 10 µL of increasing doses of SAAP-148 or halicin (in the range 0.2–102.4 µM) was added to 90 µL of the pooled bacterial suspension and incubated for 4 h at 37 °C (and 200 rpm). As a control, bacteria were exposed to PBS without SAAP-148/halicin. Afterward, the number of viable bacteria was determined microbiologically. To exclude the possibility that persisters surviving the treatment were missed, the incubation on the agar plates was prolonged for up to 5 days.

4.11. Assay for Bacterial Endotoxin Neutralization Capacities of SAAP-148 and Halicin

Approximately 15 µL of UV-inactivated (for 20 min; ChemiDoc, Bio-Rad, Hercules, California, USA) bacteria (1×10^7 CFU/mL in PBS) were mixed with 15 µL of SAAP-148 or halicin at final concentrations ranging from 1 to 1000 nM in a 96-well polypropylene V-bottom microplate and incubated for 2 h at 37 °C. Thereafter, 120 µL of Roswell Park Memorial Institute medium (RPMI, Thermo Fisher, Paisley, UK) was added to each well to dilute the samples 5×. Next, 5 µL of this mixture was added to 195 µL of 5× diluted heparinized blood obtained from healthy donors, and the mixture was incubated for 20 h at 37 °C under 5% CO_2. Subsequently, the plates were spun for 5 min at 1200 rpm; the supernatants were aliquoted and stored at −20 °C until further analysis. The level of the pro-inflammatory cytokine IL-12p40 in these supernatants was quantified using the BioLegend Elisa max deluxe set (BioLegend, San Diego, CA, USA) according to manufacturer's instructions, and the optical density (OD) at 450 nm was measured on a Spectramax i3X (Molecular Devices, Wokingham, UK).

4.12. Hemolysis Assay

The hemolytic activities of the peptide SAAP-148 and halicin were investigated. Briefly, freshly drawn citrate blood, from healthy volunteers, was washed three times with PBS by centrifugation at 1000 rpm for 10 min and then resuspended in PBS to prepare an erythrocyte suspension of 20% (v/v). Subsequently, 25 µL of PBS containing SAAP-148 or halicin (at final concentrations ranging from 12.8 to 204.8 µM) and 2% (v/v) erythrocyte suspension were mixed with 50 µL of PBS or pooled human plasma in a 96-well polypropylene V-bottom plate (Greiner bio-one), shaken for 10 sec at 300 rpm, and incubated for 1 h at 37 °C. DMSO and 5% (v/v) Triton-X (Fluka Chemie, Buchs, Switzerland) were used as controls for 0% and 100% hemolysis, respectively. After centrifugation for 3 min at 1200 rpm, the supernatant was carefully transferred to new 96-well, polypropylene flat-bottom plates, and the hemoglobin release was determined by measuring the OD at 415 nm. The percentage of hemolysis was calculated as $(OD415_{sample} - OD415_{0\mu M})/(OD415_{TritonX} - OD415_{0\mu M}) \times 100\%$, where $OD415_{sample}$ is the optical density of the peptide/halicin, $OD415_{0\mu M}$ is the optical density of the negative control, and $OD415_{TritonX}$ is the optical density of the positive controls. Hemolysis $\leq 5\%$ was considered as safe.

4.13. Statistical Analysis

Statistical analysis was performed using GraphPad Prism software version 6.0 (GraphPad Software, San Diego, CA, USA) and Kruskal–Wallis and Mann–Whitney U tests to determine the significance of the differences between the values for the peptide- and/or halicin-exposed and control samples. All the data were presented as mean; $p \leq 0.05$ was considered as statistically significant.

5. Conclusions

In conclusion, the present work revealed that the two novel antimicrobial agents, i.e., the synthetic peptide SAAP-148 and halicin, are promising candidates to fight CRIs owing to their high broad-spectrum, antibacterial, anti-biofilm, anti-persister, and anti-inflammatory activities in vitro; but importantly, their ability to act synergistically on biofilm eradication, where their combined effect is superior to their individual effects. This work provides a strong basis for further studies (using a human bladder model and animal models of catheter-related infections and for transcriptomic studies) for the clinical use of SAAP-148 and halicin to treat CRIs caused by Gram-negative bacteria. In addition, the design of more potent and safer therapeutic antimicrobial agents using several strategies, like chemical modification, nanotechnology-based delivery systems, and computer-aided design, is an excellent way to overcome the drawbacks of antimicrobial agents (by reducing their toxicities and improving their antimicrobial activities).

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/antibiotics12121743/s1: Table S1: Antibiotics tested in this study; Table S2: Target genes and primers used in this study; Table S3: PCR programs for virulence gene detection; Table S4: Concentrations of SAAP-148 and halicin tested in checkerboard assays. References [139–147] are cited in the Supplementary Materials.

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Review

Medical Device-Associated Biofilm Infections and Multidrug-Resistant Pathogens

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Abstract: Medical devices such as venous catheters (VCs) and urinary catheters (UCs) are widely used in the hospital setting. However, the implantation of these devices is often accompanied by complications. About 60 to 70% of nosocomial infections (NIs) are linked to biofilms. The main complication is the ability of microorganisms to adhere to surfaces and form biofilms which protect them and help them to persist in the host. Indeed, by crossing the skin barrier, the insertion of VC inevitably allows skin flora or accidental environmental contaminants to access the underlying tissues and cause fatal complications like bloodstream infections (BSIs). In fact, 80,000 central venous catheters—BSIs (CVC-BSIs)—mainly occur in intensive care units (ICUs) with a death rate of 12 to 25%. Similarly, catheter-associated urinary tract infections (CA-UTIs) are the most commonlyhospital-acquired infections (HAIs) worldwide.These infections represent up to 40% of NIs.In this review, we present a summary of biofilm formation steps. We provide an overview of two main and important infections in clinical settings linked to medical devices, namely the catheter-asociated bloodstream infections (CA-BSIs) and catheter-associated urinary tract infections (CA-UTIs), and highlight also the most multidrug resistant bacteria implicated in these infections. Furthermore, we draw attention toseveral useful prevention strategies, and advanced antimicrobial and antifouling approaches developed to reduce bacterial colonization on catheter surfaces and the incidence of the catheter-related infections.

Keywords: nosocomial infections; biofilms; medical device; pathogens; bloodstream infections; urinary tract infections

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1. Introduction

Every year, millions of catheters are implanted by health services to improve the management of acute and chronic diseases in adults and pediatric patients [1–3]. Unfortunately, their use inevitably allows patient's own flora or accidental environmental contaminants to access the underlying tissues and cause fatal complications [4–6]. About 60 to 70% of nosocomial infections (NI) are linked to medical devices [7]. The main complication results from the ability of microorganisms to adhere to surfaces and form biofilms [8], which protects them and helps them to persist in the host [9].Indeed, biofilms act as a protection barrier against antimicrobial agents thereby leading to therapeutic failure and increased mortality and morbidity rates [3,10,11]. Moreover, in the biofilm, there is a small subpopulation called persister cells which are characterized by increased tolerance to antimicrobials.Once the antibiotic is removed, surviving persisters are able to re-grow causing infections [12]. Several studies demonstrated that persister cells are strongly involved in chronic infections and their recalcitrance in clinical, making the antibiotic treatment innefective and biofilm eradication impossible [13–16].

In fact, catheter associated bloodstream infections (CA-BSIs) are an important cause of hospital-acquired infections originating from an intravenous catheter and associated with

morbidity, mortality, and hospital cost [17]. Central venous catheters (CVCs) are among the most widely used medical devices in critically ill patients. However, central line-associated bloodstream infections (CLA-BSIs) are the most common complications which are usually associated with the use of these CVCs [4], causing an increase in the rate of morbidity and mortality in health establishments as well as the length and costs of the stay [18]. In fact, 80,000 CVC-associated bloodstream infections (CVC-BSIs) mainly occur in intensive care units (ICUs) with a death rate of 12 to 25% [19]. Concerning peripheral venous catheters (PVCs), it has been estimated that 30 to 80% of hospitalized patients have a PVC in place during their hospitalization [20] and more than a billion PVCs are used each year around the world [21]. Among the side effects observed when a PVC is used are the following: phlebitis, partial dislodgement, accidental removal, occlusion, infiltration (fluid moving into surrounding tissue), and rarely, infections [22,23]. The incidence of bloodstream infections associated with peripheral venous catheters (PVC-BSIs) is generally low, with a rate of 0.1% of short catheters inserted (0.5 episodes per 1000 days of intravascular catheter) [24], unlike the incidence of CVC-BSIs which is 2.7 episodes per 1000 days of intravascular catheter [20]. Moreover, VC can be easily colonized by pathogenic microorganisms which lead to the formation of biofilms, other potential sources of BSIs [25]. Biofilms formed on CVCs were first described in 1982, during an epidemic of *Staphylococcus epidermidis* BSI [26]. Since that day, several studies have confirmed the involvement of biofilm in the pathogenesis of CVC-related infections and their importance [27]. Additionally, 81% of all vascular catheters that were placed in situ for 1–14 days were reported to be colonized by bacteria in the biofilm [28]. Biofilm colonization of intravenous catheters continues to affect healthcare settings [29]. Several factors increase the risk of catheter infections such as patient immunodeficiency, length of prolonged catheterization, catheter material, anatomical site of catheter insertion, poor hygiene, poor catheter insertion, and handling methods [30]. It has been reported that the incidence of bacteremia associated with PVCs is lower than that of bacteremia associated with CVCs. However, the duration of PVC insertion is 15 times longer than that of CVC insertion; for this, the number of PVC-BSIs is high due to the high number of patients who have a PVC [20,31].

Similarly, catheter-associated urinary tract infections (CA-UTIs) are the most commonly hospital-acquired infections worldwide [32]. These infections represent up to 40% of nosocomial infections. Also, 70% of UTIs are associated with urinary catheters (UC) and approximately 20% of hospitalized patients have a UC, especially those in ICUs [33]. Despite the high risk of acquiring infections with multidrug-resistant (MDR) opportunistic pathogens, most cases of catheter-associated bacteriuria are asymptomatic. However, when an episode of CA-UTI becomes symptomatic, the resulting sequelae can range from mild (fever, urethritis, and cystitis) to severe (catheter encrustation, bladder stones, pyelonephritis, endotoxic shock, and bacteremia). Left untreated, these infections can lead to urosepsis and death [34,35]. Indeed, for each day that a urinary catheter is in situ, there is a 3–8% incidence of bacteriuria, and in the majority of cases, long-term catheterization results in continued bacteriuria and symptomatic CA-UTI [36]. For an infection to be classified as a CA-UTI, a patient must have the following: (i) a urinary catheter implantated for more than 48 h; (ii) a symptom such as fever, pain, suprapubic tenderness, urinary frequency or urgency or dysuria; and (iii) urine culture with $\geq 10^5$ CFU/mL of a bacterial species [37]. However, there is much controversy over the CFU/mL cut-off in samples taken from a urinary catheter and several authorities consider that a number (greater than or equal to) $\geq 10^3$ CFU/mL is indicative of a true CA-UTI [38–40]. Moreover, other host factors such as female gender, older age (i.e., age > 50 years old), diabetes mellitus, faecal incontinence, immunocompromised status; healthcare factors such as lack of systemic antibiotics, catheter insertion outside the operating room, prolonging the duration of catheterization, and poor quality of catheter care [40–42] increase the risk of CA-UTIs. CA-UTI is linked with biofilm formation along the surface of the catheter [36]. Indeed, the presence of a UC facilitates bacterial colonization due to the development of a conditioning film of host proteins which provides bacteria with an ideal substrate for fixation [43].

In view of all this, there is an urgent need to develop novel strategies to fight medical device-associated biofilms. Despite several studies having been conducted in this field, many challenges still remain. This review provides an overview on the medical device-associated biofilm infections (mainly venous catheter-associated bloodstream infections and catheter associated-urinary tract infections), the biofilm development process on these devices, and the most MDR-bacteria implicated in these infections with their virulence factors. Furthermore, the current review highlights the different prevention strategies and the most effective approaches using antimicrobial coating and antifouling methods, to reduce medical device colonization and the incidence of their related infections.

2. Hospital-Acquired Infections

Hospital-acquired infections (HAIs) or nosocomial infections (NI) are defined as infections which were neither present nor incubating during the patient's hospitalization and were acquired after 48 h of hospitalization. These infections increase patient morbidity and mortality, prolong their hospital stays, and represent a massive additional financial burden for health structures [44]. The severity of infection and its incidence is much higher in patients in burn units, intensive care units, organ transplant receivers, and with newborns due to their immunological status [45]. In addition to the problems associated with nosocomial infections, antibiotic resistance and the emergence of MDR-bacteria is a serious global problem, due to the uncontrolled administration of drugs [46]. These HAIs are often the result of the use of invasive procedures such as the location of temporary indwelling devices (VCs, UCs, endotracheal tubes, and wound drains) or are associated to the placement of cardiovascular or orthopedic implants during a surgical intervention [47]. They include a wide range of infections such as catheter related infections (CRIs), CA-UTIs, and ventilator associated pneumonia (VAP) [48]. These infections are generally designed as "Medical Device-Associated Biofilm Infections" [3]. Several Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*) and Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Enterococcus faecalis*) bacteria are involved in the onset of NIs [49,50]. Their ability to form a biofilm makes the treatment of these infections more complicated [51].

In fact, it is well known that biofilms have a significant impact in medicine through the development of HAIs [52] and it is estimated that bacterial biofilms are involved in 65% of NIs and in more than 80% of chronic infections [53]. Treatment of these infections requires administration of high dose antibiotics and/or replacement of the device, which are both ineffective due to the antibiotic resistant strains and the high risk of re-infection on the new device [51].

3. Biofilm Formation on Medical Devices

Bacteria have always been studied in the laboratory as planktonic microorganisms. However, most bacteria live in multicellular communities called biofilms [54]. The biofilm was observed for the first time in the 17th century by Anthony van Leeuwenhoek through his microscope in his own mouth where he observed aggregated microorganisms on his teeth and tongue [55]. A biofilm is a highly structured bacterial community attached to a surface and protected by a self-produced extracellular polymeric matrix [56–58]. This matrix is mainly composed of proteins, polysaccharides, and extracellular DNA (eDNA). Furthermore, the biofilm matrix is highly hydrated and contains up to 97% water, mainly responsible for nutrient transport within the biofilm [53,59]. Bacterial adhesion occurs on a pre-conditioning film formed on the surface after deposition of organic, inorganic, and cellular components (e.g., fibronectin, fibrinogen, laminin, collagen, polysaccharides) found in the environment surrounding the medical device, constituting a base on which the biofilm will develop [51,57]. The bacteria interact with the components of this surface through appendages, attractive forces, or adhesins [57]. The biofilm formation on a medical device proceeds as follows: (i)Transport of bacterial cells to the surface—the bacteria can be transported to the medical device either by diffusion (Brownian motion),

convective flow or active movement (motile bacteria) [60]. Bacterial transport can also be induced by chemotaxis due to the presence of diffusible chemical gradients which form from various chemical stimuli or the degradation of components (eg. aspartate, glucose, galactose) [60,61]. However, if the surface is unsuitable for bacterial adhesion, the bacteria return to the planktonic state [11]. (ii) Reversible attachment—this first step of the bacterial adhesion process to the medical device is the transport of cells to the device [61]. The initial attraction mainly involves non-specific physical interactions such as Van der Waals attractive forces, electrostatic forces (attractive or repulsive), hydrophobic interactions, Brownian motion, and gravitational forces [62]. Furthermore, due to the negative charge of their cell membrane, bacteria are subjected to repulsive electrostatic and repulsive hydrodynamic forces when they are near the medical device. In order to overcome these two repulsive barriers, bacteria typically use cellular appendages, such as flagella or pili [63,64]. This initial binding of bacteria to the medical device is important to make irreversible adhesion possible [60]. (iii) Irreversible attachment—this step is characterized by stronger induced cell–surface interactions and shorter distances which allow adhesins exposed to the cell surface to form a bond with the biomaterial [65]. Gene expression which encodes for bacterial surface structures including fimbriae, pili, lipopolysaccharides, and slime will also begin to strengthen adhesion promoting biofilm formation [65,66]. (iv) Cell proliferation and formation of microcolonies:—once the bacteria have become attached to the surface of the medical device and stabilized, the cells will proliferate rapidly and produce intercellular adhesins to form microcolonies. In this step, the QS "quorum sensing" communication system is activated when the bacterial density reaches a threshold [11,57,67]. The gene expression of components required for a biofilm matrix such as polysaccharides, proteins, eDNA, and lipids is also activated [68]. (v) Device surface colonization—the cells continue to proliferate and additional surrounding planktonic cells are also incorporated into the biofilm [69]. (vi) Biofilm maturation—the multilayers continue to form, inducing an increase in thickness, thereby allowing the transition from a two-dimensional arrangement to a three-dimensional arrangement (mature biofilm) [67] also called "mushroom" structure [70]. Channels filled with water are formed in the biofilm allowing the transport of nutrients, signaling molecules, and elimination of waste [53,71]. (viii) Biofilm dispersion—once the biofilm is mature, the planktonic bacteria detach from the biomaterial due to hydrolase enzymes then migrate and colonize new surfaces, spreading the infection [11,12]. The main steps are illustrated in Figure 1 below.

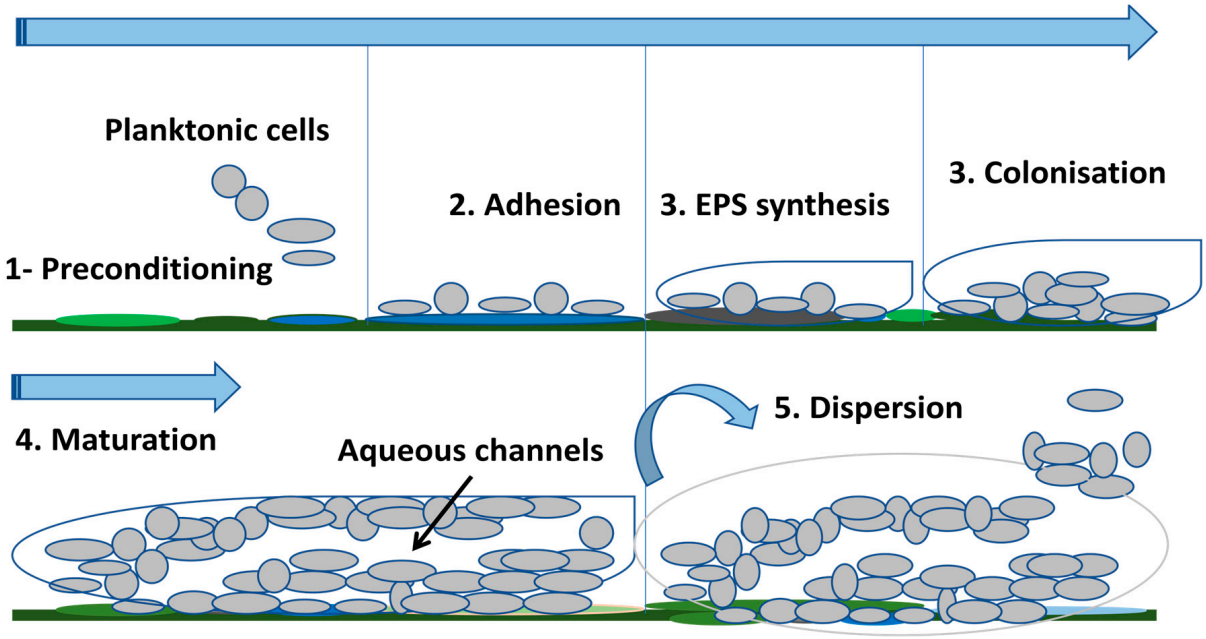


Figure 1. Biofilm formation steps on medical devices.

4. Most Common Pathogenic Bacteria Involved in Medical Device-Associated Biofilm Infections

4.1. Escherichia coli

Escherichia coli is responsible for a wide variety of community and HAIs such as UTIs and BSIs with increasing antimicrobial resistance rates [72,73]. Indeed, in addition to the global emergence of resistance to carbapenems, the significant increase in the prevalence of quinolones-resistant *E. coli* strains has also been reported in several countries limiting the treatment choice for these bacterial infections, thereby constituting a real problem for public health [74,75]. Uropathogenic *E. coli* (UPEC) is the bacterium mostly involved in 80 to 90% of UTI cases [76,77] and in 40% of hospital acquired UTIs [78]. These strains have become more resistant to antibiotics with the increasing prevalence of extended-spectrum β-lactamases (ESBLs) [74,79]. *E. coli* is one of the bacteria most implicated in biofilm-related infections, particularly in CA-UTIs [80–84] and CVC-BSIs [85–88]. In fact, biofilm formation is the major cause of these infections in catheterized patients, making them hard to eradicate [89,90]. Indeed, their ability to form a biofilm is associated with the persistence and chronicity of inflammations leading to complicated and/or recurrent infections [91,92]. *E. coli* strains have an arsenal of virulence factors which contribute to adhesion, colonization, and persistence allowing the different defense mechanisms of the host to be overcome [93]. Among them, we distinguish adhesins, *fimbriae*, toxins, siderophores, etc. Table 1 summarizes the virulence factors implicated in *E. coli* pathogenesis and especially adhesion and biofilm formation. The role of virulence genes of *E. coli* in its adhesion to catheter surfaces has been reported in several studies [82,83,89,90,92,94–102]. For example, Reisner et al. [94] reported that 73% of *E. coli* strains isolated from catheterized patients expressed type 1 *fimbriae*. In a recent study of Zou et al. [90], they showed that biofilm associated genes such as iron transport systems (ferric citrate) and antigen 43 may be involved in the pathogenic CA-UTI strains. Another recent study demonstrated that the knockout of *luxS*, *fimH*, and *bolA* genes decreased EPS matrix production, which is very important in *E. coli* biofilm-associated UTIs [83].

Table 1. Main medical device-associated bacteria and their virulence factors.

Pathogen	Virulence Factor	Characteristics and Function	Reference
E. coli	Type I *fimbriae*	Encoded by *fim* operon located on the chromosome of UPEC isolates; Binding specifically to D-mannose which is found on the glycoproteins of the epithelial cells; Represents 95% of the virulence factors of *E. coli*; A major adhesin in colonization of UCs and biofilm formation during CA-UTIs.	[94,97,98,103–105]
	P *fimbriae*	Pyelonephritis-associated pili (Pap); The second important adhesin expressed by UPEC and coded by the *pap* operon; Binding to di-galactoside moiety present in the urinary tract epithelium.	[100,106,107]
	Curli Csg A	Key components of the extracellular biofilm matrix of *E. coli* in which CsgA is the major subunit of curli; Bacterial binding with fibronectin, laminin, plasminogen; Aggregation, adhesion to surfaces, and biofilm development.	[108,109]
	PGA (Poly-β-1,6-N-acetyl-D-glucosamine)	Encoded by *pgaABCD*operon; A primary component of the biofilm matrix; Attachment of *E. coli* to surfaces and autoagregation of cells.	[82,89]
	Ag 43 (Antigen 43 adhesin)	One of the major auto-transporter in *E. coli* encoded by the gene *flu*; Translocation to the outer membrane; Adhesion and auto-aggregation (cell-to-cell) facilitating the formation of the biofilm.	[89]
	Hemolysin F	Encoded by the gene *hlyF*; Over-expression of *hlyF* promotes the biosynthesis of the outer membrane vesicles (OMVs) which release toxins involved in virulence.	[110,111]
	α-hemolysin	Encoded by the gene *hlyA*; A pore-forming cytotoxin, responsible for lysis of the cell membrane of hosts (leukocytes, erythrocytes, and endothelial cells).	[112]
	Siderophores	Survival and colonization in iron-deficient sites.	[78]
	CNF-1 (cytotoxic necrotizing factor 1)	Responsible for the apoptosis of urothelial cells then increase bacterial entry to the bladder.	[89]
	LPS (Lipopolysacharide)	An endotoxin that induces septic shock caused by over-expression of pro-inflammatory cytokines.	[113]
	Capsule	Adherence to host cells; Biofilm formation; Binding to C4 binding protein (C4BP) and inhibits complement cascade; Bacterial protection from phagocytosis; Binding to catioinc antimicrobial agents; Bacterial protection from macrophage recognition.	[114]

Table 1. *Cont.*

Pathogen	Virulence Factor	Characteristics and Function	Reference
	Quorum sensing	Main *quorum sensing* systems: LuxS and SdiA, producers of autoinducer-2 molecules; Cell-to-cell communication; Role in bacterial behaviour coordination and regulation of virulence genes.	[115]
K. pneumoniae	Types 1 *fimbriae*	Encoded by *fimAICDFGHK* operon; Adhesion mediation to mannose-containing structures present on host tissue and extracellular matrix; A major role in biofilm formation on UCs, invasion and colonization of host cells, and persistence in catheters-associated infections.	[116,117]
	Types 3 *fimbriae*	Encoded by *mrkABCD* operon; Adhesion to different structures in kidney, lung tissue, endothelial and bladder epithelial cells; A major role in biofilm formation on UCs, invasion and colonization of host cells, and persistence in catheter-associated infections.	
	PGA (poly-β-1,6-N-acetyl-D-glucosamine)	Encoded by *pgaABCD* operon; Cell-cell communication; Intercellular adhesion; Adhesion to abiotic surfaces.	[118]
	Capsule polysaccharides (*magA*, *k2A* and *wcaG)*	Resistance to phagocytosis; Complement-mediated lysis inhibition and opsonization; Host defense escape.	[119,120]
	Lipopolysaccharides (*wabG*, *uge*, *ycfM)*	Inhibition of complement pathway; Inactivation of the seditious response; Block the effect of peptides via lipid A; Host defence escape.	
	Siderophores (*iutA*, *iroN*, *entB)*	Acquisition of iron from host iron-chelating proteins for survival and growth during infections; Biofilm formation; Host defence escape.	
	Quorum sensing	*QS* regulator systems: Type 2 *quorum sensing luxS*; Cell–cell communication; Intercellular adhesion and adhesion to abiotic surfaces; Bacterial behaviour coordination; Regulation of virulence genes.	[118,121]

Table 1. *Cont.*

Pathogen	Virulence Factor	Characteristics and Function	Reference
P. mirabilis	MR/P (Mannose resistant *Proteus*-like *fimbriae*)	Binding to uroepithelial cells; Attachment to urinary catheters; Biofilm formation.	[33,122,123]
	PMP (*P. mirabilis* P-like pili)		
	PMF (*P. mirabilisfimbriae*)		
	ATF (Ambient-temperature *fimbriae*)		
	UCA (Uroepithelial cell adhesin)		
	Flagella	A major role in swarming; Migration of pathogenic strains to the upper urinary tract, causing pyelonephritis; Dispersion of biofilm from urinary catheters to the urinary tract.	[33,124,125]
	Hemolysin *HpmA*	Ability to lyse erythrocytes, bladder epithelial cells, and monocytes; High cytotoxicity towards human renal proximal tubular epithelial cells (HRPTECs); Dessimination of *P. mirabilis* into the kidneys and development of pyelonephritis.	[122,126,127]
	Proteus toxigenin *Pta*	An autotransporter which promotes autoaggregation of the bacteria. Mediates lysis of bladder epithelial cells.	
	ZaPA(zinc metalloproteinases)	Degradation of immunoglobulins IgA and IgG, human β-defensin 1, and other celluar components (fibronectin, collagen); Escape immune responses during infection.	
	LPS (Lipopolysccharides)	Mediation of the induction of proinflammatory cytokine responses; Induction of apoptosis; Septic shock.	
	Iron acquisition system	Production of iron carriers to take iron from the host and use it for its survival during urinary infections.	
	Urease (a nickel-dependent metalloenzyme)	Degradation of urea into carbon dioxide and ammonia, increasing the urine pH up to 8.2; Formation of crystals, struvite (ammonium and magnesium phosphate) and apatite (calcium phosphate); Causing the obstruction of urinary catheters; Causing pyelonephritis and increasing the risk of sepsis.	[128,129]
	Quorum sensing	*QS* system regulator: luxS/luxR system; Autoinducer-1 molecules controlled by the *luxR* genes and autoinducer-2 molecules controlled by the *luxS* genes; Role in swarmingcoordination; Regulation of biofilm formation and virulence genes expression.	[125]

Table 1. *Cont.*

Pathogen	Virulence Factor	Characteristics and Function	Reference
P. aeruginosa	Flagella Type IV pili	Important role swimming, twitching, and swarming motility; Adhesion to host epithelial cells; Attachment to surfaces and biofilm formation.	[130]
	LPS (Lipopolysccharides)	Antibiotic tolerance, tissue damage, and biofilm formation; The complement system induction; Activation of inflammatory cytokines TNF-α and IL-1β; Induction of immune responses via Toll-like receptor 4 (TLR4) and cystic fibrosis transmembrane conductance regulator (CFTR); Induction of phagocytosis; Neutrophil activation for neutrophil extracellular trap (NET) releasing which contain pathogens.	[131,132]
	Exopolysaccharides (alginate, PEL and PSL)	Crucial role in initial attachment to surface; Biofilm formation, its stability and maintenance; Bacterial protection from phagocytosis and opsonization, Biofilm maturation, and prevention of antibiotic diffusion.	[133]
	OMVs (Outer membrane vesicles)	Expression of 26 OMPs of which the porin OprF is the most abundant; Transport of molecules (e.g.,toluene, siderophores, nitrates, and nitrites); Bacterial adhesion and biofilm formation; Implication in drug resistance; Protection from macrophage clearance during chronic infections; Remove of competing bacteria from the environment during infections.	[134,135]
	Siderophores (Pyoverdine and Pyochelin)	Iron chelation from transferrin and lactoferrin needed during growth and virulence.	[131,136]
	Elastase A [LasA] and B [LasB]	Degradation of elastin; Host tissues damage.	
	Protease IV	Degradation ofcomplement components, immunoglobulins, and surfactant protein; Raising of bacterial infection via fibrinogen, lactoferrin, transferrin degradation; Host tissues damage.	

Table 1. *Cont.*

Pathogen	Virulence Factor	Characteristics and Function	Reference
	toxins (Pyocyanin; T3SS effectors [ExoS, ExoT, ExoU and ExoY]; Exolysin [ExlA]; Exotoxin A [PEA]; Lipase A [LipA] and Leukocidin)	Pyocyanin: induction of oxidative stress for the host to avoid bacterial elimination; T3SS effectors: inhibition of phagocytosis and bacterial elimination, disruption of the host actin cytoskeleton, and apoptosis induction; ExlA: induction of membrane permeabilization and cell death through its cytolysin activity; PEA: inhibition of host protein synthesis by ADP ribosylation activity and stimulatation of programmed cell death; Lipase A: degradation of lipid dipalmitoylphosphatidylcholine (lung surfactant) and drug resistance mediation by interacting with alginate; Leukocidin: leukocytes swelling by increased permeability of their membrane.	
	Quorum sensing	Main*QS*systems: *Las*, *Rhl*, *Pqs* and *Iqs*; Regulationof biofilm formation and other virulence factors; Coordination of bacterial behaviour and persistance during infection.	[137,138]
A. baumannii	Csu (Chaperone-usher pili)	Encoded by *csuA/BABCDE* operon; Involved in the initial ahesion onto abiotic surfaces but not biotic surfaces.	[139,140]
	OmpA (Outer membrane protein A)	A β-barrel porin, one of the most abundant porins in the outer membrane of *A. baumannii.* Role in the virulence of *A. baumannii*, including interaction with the host, cytotoxicity, apoptosis, and biofilm formation.	[139,141–143]
	Bap (Biofilm-associated protein)	Formation of water channels; Maitainingthe structure and integrety of biofilm; Biofilm formation on abiotic and biotic surfaces.	[139,144]
	PNAG (Poly-N-acetyl β-1-6 glucosamine)	Major component of the *A. baumannii* biofilm matrix and encoded by the *pgaABCD* operon; Role in the integrity of the biofilm; Tolerance to desiccation stress; Incredible persistence in natural environments and care facilities.	[144,145]
	Type V secretion systems	Transport exoproteins; Transport mobile genetic elements; Role in bacterial competition; Biofilm formation on abiotic and biotic surfaces.	
	Phospholipases C and D	Hydrolytic activity towards phosphatidylcholine; Hemolytic activity against erythrocytes.	
	Capsule	A protection barrier. Resistance to some antibiotics. Regulation of the *K locus* genes for exopolysaccharides production, important for biofilm formation.	

Table 1. *Cont.*

Pathogen	Virulence Factor	Characteristics and Function	Reference
	Iron-chelator proteins	Uptake of iron from host environnement in iron deficiency conditions.	[140]
	Quorum sensing	Two-component regulatory system: AbaI/AbaR system Regulation of several virulence factors such as biofilm formation and motility.	[139]
S. aureus	PIA (Intracellular adhesion polysaccharide) or PNAG (o poly-N-acetyl β-1-6 glucosamine).	Encoded by the *icaADBC* operon; Important in cell-to-cell adhesion, adhesion to surfaces, biofilm formation; Antimicrobial resistance; Immune evasion; Bacterial protection from phagocytosis.	[146–148]
	FnBPA and FnBPB (Fibronectin-binding proteins)	Categorised as "microbial surface component recognising adhesive matrix molecules (MSCRAMM)"; Implication in binding host matrix components (fibronectin, fibrinogen, collagen, elastin, laminin); Initial cell attachment and/or biofilm formation; Implication in colonization; Immune evasion.	[146,149,150]
	ClfA and ClfB (Clumping factors)		
	Can (Collagen adhesin)		
	EbpS (Elastin binding protein)		
	Fib (Fibrinogen binding protein)		
	Eno (Laminin-binding protein)		
	SdrC, SdrD, SdrE (Serine aspartate repeat proteins C, D, and E)		
	Atl (Autolysin)	Primary attachment through non-specific hydrophobic interactions with uncoated surfaces; Bindin to host extracellular matrix proteins and involvment in cell separation during cell division.	[151,152]
	Bap (Biofilm-associated protein)	Contributionin initial adhesion to abiotic surfaces; Induction of strong intercellular adhesion.	[153,154]
	Quorum sensing	Global regulatory systems [accessory gene regulator (*agr*), staphylococcal accessory element (*sae*), the staphylococcal accessory regulator A (*sarA*)]; Regulation of the expression of virulence factor secretion and biofilm formation.	[155]
S. epidermidis	PIA (Intracellular adhesion polysaccharide)	Adhesion and biofilm accumulation.	[156]
	AtlE (Autolysin E)	Attachment to plastic surfaces.	[157]
	Bap homolog protein Bhp	Adherence to a polystyrene surface, intercellular adhesion, and biofilm formation.	
	Ssp-1,Ssp-2 (Staphylococcal surface proteins 1 and 2)	Cell-to-cell adhesion; Biofilm formation.	

Table 1. *Cont.*

Pathogen	Virulence Factor	Characteristics and Function	Reference
	Serine-aspartate repeat protein G (SdrG/Fbe) binding to fibrinogen	Adhesion to the host proteins (fibrinogen, collagen, fibronectin); Adhesion to abiotic surfaces.	[158]
	SdrF (Serine-aspartate repeat protein F) binding to collagen		
	Extracellular matrix-binding protein (Embp)		
	Phenol-soluble modulins (PSMα, PSMδ, PSMε, δ-toxin [PSMγ], and PSMβ [PSMβ1, PSMβ2])	Acquisition of the characteristic three-dimensional structure-like mushrooms; Role in biofilmdispersion.	[157,159]
	Homolog of the SspB	Role in the degradation/dispersion of the biofilm.	[157]
	Homolog of SspA V8		
	Metalloprotease SepA		
	Nucleases		
	Quorum sensing	Two key systems: the *agr* and the *sar* regulators; Expression/repression of virulence genes in a coordinated manner during infection.	[160]
En. faecalis	Esp (Enterococcal surface protein)	Primary adhesion in UTIs; Colonization of the urinary tract.	[161,162]
	Asa1 (Aggregation substance)	Adhesion to host cells and bacterial aggregation.	
	Collagen binding protein (Ace)	Adhesion to extracellular matrix and type 1 collagen.	
	EfaA(*En. faecalis* endocarditis antigen A)	Adhesion to biotic and abiotic surfaces.	
	Epa (Enterococcal polysaccharide antigen)	Colonization, translocation through epithelial cells, bacterial adhesion, biofilm formation, and antibiotic resistance.	[163]
	Ebp A,B,C (Endocarditis and Biofilm-Associated Pili)	Important in initial attachment, biofilm formation, and endocarditis.	[164,165]
	CylA (Cytolysin A)	Killing other bacteria (especially Gram-negative bacteria) and eukaryotic cells (red blood cells); Biofilm formation.	[162,165,166]
	Hyl (Hyaluronidase)	Degradation of hyaluronic acid to permeabilize host tissues; Induction of autoimmune diseases.	

Table 1. *Cont.*

Pathogen	Virulence Factor	Characteristics and Function	Reference
	GelE (Gelatinase)	Degradation of the collagen adhesion protein (Ace) which contributes in colonization and biofilm formation; Degradation of gelatin, collagen, fibrin, fibrinogen, hemoglobin, and complement components (C3, C3a, C5a); Cell lysis.	
	SprE (Serine protease)	Degradation of casein; Release of eDNA.	
	Quorum sensing	Regulator system: the Fsr (fecal streptococci regulator) *quorum-sensing* system, encoded by *fsrA*, *fsrB* and *fsrC* genes; Regulation of communication through peptide pheromones cpd, cob, and ccf; Control of biofilm formation via regulation of gelatinase production.	[167]

4.2. Klebsiella pneumoniae

Klebsiella pneumoniae is considered to be one of the most important opportunistic pathogens responsible for NIs including sepsis, soft tissue infections, pneumonia [168], and UTIs, which are the most common worldwide [169]. *K. pneumoniae* is implicated in 6–17% of opportunistic UTI cases mainly linked to bacterial adhesion in the inner and outer surfaces of the urinary catheter [170,171]. Moreover, it is the second pathogen involved in BSIs, after *E. coli* [172]. The prevalence of NIs due to *K. pneumoniae* has been reported to be approximately 10% worldwide [173]. Several studies revealed the implication of *K. pneumoniae* in HAIs, especially in catheter-related infections (CRIs) [18,85,172,174–179]. Since this bacterium is commensal in humans, gastrointestinal colonization represents the major source of transmission and the development of infections towards other sites [180]. The global antibiotic resistance rate of *K. pneumoniae* is approximately 70% with mortality rates ranging from 40% to 70% [181] and the emergence and spread of MDR-strains of *K. pneumoniae* have becomea real global problem [182]. In fact, this bacterium is able to acquire resistance genes such as ESBLs or carbapenemases (resistance to cephalosporins or third-generation carbapenems), limiting treatment options for infections [168], causing serious or even fatal infections, while also increasing the length of hospitalization and the costs of processing [183]. Moreover, carbapenem-resistant *K. pneumoniae* are the major cause of BSIs with high rates of mortality and morbidity in the world [179]. Another group of *K. pneumoniae* strains called "hypervirulent", able to express acquired virulence factors, has also emerged causing serious community-acquired infections [184]. Many *K. pneumoniae* isolates are able to form biofilms, resulting in increased impermeability to antibiotics causing treatment failure [185]. For this, the World Health Organization (WHO) classifies this bacterium among the high priority species and encourages the development of new antimicrobial molecules in order to counter its antibiotic resistance [169]. *K. pneumoniae* has several virulence factors, including types 1 and 3 *fimbriae*, capsule polysaccharides, LPS, *quorum-sensing*, and PGA [118] used to escape host immune defenses, biofilm formation, and to persist during infection [186].The adhesins of *K. pneumoniae* allow the establishment of strong biofilms [116,187–194].An in vitro study reported the improvement of biofilm formation on UCs by the presence of types 1 and 3 *fimbriae* in a bladder model [116]. In a recent study, the results showed that 87.5%, 46.4%, and 53.6% of strains harbored *fimH*, *mrkA*, and *mrkD* fimbrial genes, respectively [193]. Other genes have been reported to be involved in biofilm formation [119,187,191,194–197]. The main virulence factors of *K. pneumoniae* are summarized in Table 1.

4.3. Proteus mirabilis

Proteus mirabilis is an opportunistic pathogen that causes infections in immunocompromised individuals but also NIs, including wound infections, blood infections, and mainly UTIs [50]. It is recognized as the main cause of CA-UTIs, and in the USA, 3% of all HAIs and 44% of CA-UTIs are linked to this bacterium [126]. In fact, *P. mirabilis* is considered the third most common cause of UTIs and the second most common cause of CA-UTIs in long-term catheterized patients [198]. Furthermore, UTIs, particularly CA-UTIs, caused by *P. mirabilis* generate serious complications such as the formation of bladder and kidney stones, permanent kidney damage, or even bacteremia/sepsis which can be fatal for patients [199]. In addition, trauma to the urethra and bladder mucosa may also occur during catheter removal [124]. All these complications are due to the capacity of *P. mirabilis* to form crystalline biofilms, leading to an obstruction [126]. *P. mirabilis* was reported as a common agent causing UTIs and CA-UTIs in several studies [122,126,198,200–203]. This commensally bacterium colonizing the perianal area of patients, easily penetrates into the bladder after implantation of the catheter, and thus adheres to it [204]. In addition, in recent years, *P. mirabilis* strains have become increasingly resistant to drugs, especially, the isolates producing ESBLs, leading to therapeutic failure, thereby constituting a worldwide problem for public health [123,205–207].

Proteus mirabilis has a panel of virulence factors (Table 1); however, its pathogenicity is exacerbated by biofilm formation, making the infection worse [208]. In fact, this uropathogen has been shown to have a great ability to form biofilm and is involved in the encrustation and blockage of UCs—a common complication in patients with long-term indwelling urinary catheterization—and is a major cause of morbidity and mortality in CA-UTIs [124,129]. Numerous adhesins, including MR/PM *fimbriae*, MR/KH, PM *fimbriae*, urethroepithelial adhesin (UCA), and ambient-temperature *fimbriae* (ATF) have been associated to *P. mirabilis* adhesion onto UCs and biofilm formation during CA-UTIs [126,203,209–211].

4.4. Pseudomonas aeruginosa

Pseudomonas aeruginosa is one of the six bacterial pathogens of the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *K. pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa*, and *Enterobacter* species) known for their high antibiotic resistance and increased virulence, which pose big challenges to treatment worldwide [136,212,213]. In fact, the resistance of this pathogen to many antibiotics, particularly resistance to carbapenems, constitutes a serious threat to global public health and increases morbidity and mortality rates, particularly in ICUs [137,214]. This is why the WHO designated this MDR-bacterium as one of the priority antibiotic-resistant pathogens for which new antibiotics are urgently needed [136]. *P. aeruginosa* is one of the most opportunistic pathogens causing fatal acute or chronic infections in immunocompromised hosts. Indeed, it is one of the most common pathogens found in hospitals and is responsible for more than 50% of NIs [213]. The most HAIs caused by *P. aeruginosa* are VAP, UTIs, CA-BSIs, burn wound infections, skin and soft tissue infections, surgical site infections, and ocular infections [131,215]. Moreover, *P. aeruginosa* has the ability to form biofilms during infections causing increased resistance to antibiotics and the persistence of NIs, which are considered fatal for patients [216]. It is estimated that *P. aeruginosa* is responsible for 28% of device-related infections [217]. A rate of 11.5% of *P. aeruginosa* strains was recovered from UCs and all of them were MDR [217]. The implication of *P. aeruginosa* in CA-UTIs [218–221] and in CL-BSIs [85,87,88,175,222] has been widely reported. Importantly, a recent international study on infections in ICU-patients demonstrated that *P. aeruginosa* was responsible for 23% of all infections acquired in ICUs, with the respiratory source being its main site [223]. These bacteria have an impressive arsenal of virulence factors which contributes to their pathogenesis (Table 1). Flagella and pili are virulence factors involved in motility as well as bacterial adhesion, which is the starting point of infections [214,224–227]. Also, the EPS Psl and Pel, major components of the biofilm matrix of *P. aeruginosa*, significantly contribute in bacterial adhesion to the catheter surface, cell–cell aggregation, and stability of the biofilm structure [217]. Several reports showed the importance of these EPS [219,228,229].

4.5. Acinetobacter baumannii

Acinetobacter baumannii is a nosocomial pathogen that is responsible for a large number of infections in humans, including endocarditis, UTIs, meningitis, pneumonia (in mechanically ventilated patients), and sepsis [141]. The incidence rate of *A. baumannii* infections is estimated to be approximately one million cases per year in the world, with high mortality rates, especially in critically ill patients [230]. The high prevalence of MDR-*A. baumannii* has become a serious situation in a hospital setting. One of the major factors responsible for the chronicity and persistence of infections and resistance to antibiotics of *A. baumannii* is its ability to colonize and form a biofilm on biotic and abiotic surfaces (e.g., vascular catheters, cerebrospinal fluid shunts, or Foleys catheter) [231,232]. In addition to MDR-*A. baumannii*, extremely-drug resistant (XDR) and pan-drug resistant (PDR) isolates have also been reported worldwide [230]. Most *A. baumannii* infections occur in ICU-patients and account for up to 20% of ICU-infections worldwide [141,233], with an increase in mortality rates (30% to 75%) [234]. Moreover, their ability to acquire resistance to antibiotics and persistence in the environment are the main factors contributing to their survival in the hospital environment [235]. CRIs associated with MDR-*A. baumannii* biofilms have

been widely reported [236–243]. *A. baumannii* possess several adhesins (Table 1), which contribute to biofilm formation and bacterial colonization on medical devices [230]. Several reports have shown the presence of *omp*A (detection from 81 to 100%), *csuE* (detection from 80 to 100%), and *bap* (detection from 43 to 91.4%) in clinical *A. baumannii* strains producing strong biofilms [232,244–248]. In a recent study, Kasperski et al. [249] reported that 72% of strong biofilm producer *A. baumannii* strains harbored four genes associated with biofilm formation (*bap*, *bfmS*, *csuE*, and *ompA*), showing their implication in bacterial adhesion on surfaces.

4.6. Staphylococcus aureus

Staphylococcus aureus is a clinical pathogen that causes infections in both humans and animals, ranging from mild infections to severe invasive life-threatening infections [250]. This organism is also the main agent involved in NIs due to its incredible ability to adhere to the surface of medical devices and form a biofilm, which often leads to chronic infections such as osteomyelitis, endocarditis, CF, catheters infections, prostheses infections, and other medical device-associated infections [251–253]. *S. aureus* is one of the major pathogens responsible for nosocomial blood infections with an incidence estimated between 10–30 cases/100,000 persons/year and associated with mortality rates ranging between 15 and 40% [254]. In addition to this, the global emergence of methicillin-resistant *S. aureus* (MRSA) is a major public health concern given its high virulence and therefore, treatment failure is unavoidable [155], increasing mortality and morbidity in patients [251]. It is important to note that the *mecA* gene is the genetic determinant of methicillin resistance in MRSA, which is located on the mobile genetic element called staphylococcal cassette chromosome mec (*SCCmec*) [255]. It has a remarkable arsenal of virulence factors (Table 1) including toxins, proteases, nucleases, but also many proteins anchored in the cell wall which important factors are allowing it to adhere to tissues, to surfaces, to form a biofilm, and to escape the host's immune defense [256,257]. It has been reported that the mortality rates associated with MRSA bacteremia were higher than those associated with methicillin-sensitive *S. aureus* (MSSA) bacteremia [258]. CR-BSIs due to *S. aureus* are considered to be the most fearful of infections. Indeed, Mandolfo et al. [259] identified 113 CR-BSIs caused by MRSA (47.5%) and MSSA (52.5%) in hemodialysis patients. In the study of Bonnal et al. [260], 56% of *S. aureus* in PVC-BSIs and 34% of *S. aureus* in CVC-BSIs were identified. Recently, Pinto et al. [261] identified *S. aureus* as the main causative agent of CR-BSIs with a rate of 24.1%. Other studies [85,175,222,258,262–266] also reported implication of *S. aureus* in CR-BSIs. *S. aureus*, especially MRSA, also constitutes a serious problematic pathogen in CA-UTIs [267]. In fact, *S. aureus* can colonize the urinary tract via urinary catheterization causing ascending UTIs. The presence of MRSA complicates the situation, extending the length of hospital stay [268]. This pathogen causes approximately 0.2–4% of UTIs and is more often found in patients in long-term care and with long-term catheters [269]. Several studies have shown the implication of *S. aureus* in CA-UTIs [267,270,271].

Various reports showed the implication of virulence genes in *S. aureus* healthcare infections including CRIs. The major factor in staphylococcal biofilm formation is the polysaccharide intercellular adhesin (PIA) or poly-N-acetyl β-1-6 glucosamine (PNAG), encoded by the *icaADBC* operon [272–274]. This polysaccharide plays an important role in colonization, biofilm formation and biofilm-related infections, antimicrobial resistance, immune evasion, and phagocytosis [146]. Several studies reported a positive correlation between the presence of *icaAD* genes and the ability of *Staphylococcus* strains to produce a biofilm [275–277]. However, Pinto et al. [261] showed no relationship between the presence/absence of the *ica* operon and biofilm formation on CVCs. Another recent study showed that 4/6 *S. aureus* strains, that did not carry *ica* genes, were strong biofilm producers [278]. This indicates that other genes may be involved in biofilm formation. Indeed, *S. aureus* contains also a range of proteins categorised as "microbial surface component recognising adhesive matrix molecules (MSCRAMM)" [146,149] such as fibronectin-binding proteins (FnBPA and FnBPB), clumping factors (ClfA and ClfB), collagen adhesin (Can),

elastin binding protein (EbpS), fibrinogen binding protein (Fib), laminin-binding protein (Eno), and serine aspartate repeat proteins C, D, and E (SdrC, SdrD, SdrE) [279,280], which have been implicated in binding host matrix components (fibronectin, fibrinogen, collagen) to initiate cell attachment and/or biofilm formation [150]. The association between the MSCRAMMs adhesins ClfA/B, FnbA/B, and Cna with bacteremia and catheter-related bacteremia has also been reported [281,282]. Similarly, Walker et al. [267] reported that the clumping factor ClfB, interacting with fibrinogen, facilitates colonization of the UC and bladder leading to infection in mice and humans in a CA-UTI model.The expression of these virulence factors and biofilm formation are regulated by global regulatory systems such as accessory gene regulator (*agr*), staphylococcal accessory element (*sae*), and also by staphylococcal accessory regulator A (*sarA*) [155]. Pérez-Montarelo et al. [283] reported that more than half of MRSA-related bloodstream isolates belong to the accessory gene regulator (*agr*) group II.

4.7. Staphylococcus epidermidis

Staphylococcus epidermidis is one of the most ubiquitous opportunistic pathogens [284] that causes serious infections in immunocompromised patients, especially those associated with the presence of invasive medical devices (e.g., VC and artificial heart valves) [156]. However, this bacterium rarely causes CA-UTIs and rarely pyelonephritis (without an indwelling urinary device) [285]. It is estimated that 30% of CA-BSIs are due to *S. epidermidis* strains [286]. *S. epidermidis* isolates were implicated in several CA-BSIs. A recent report revealed that this species was the most causative agent in CA-BSIs (13.3%) in the emergency department [287]. A prospective observational study conducted by Pinto et al. [261] showed that *S. epidermidis* was the most etiological agent of CR-BSI. A similar retrospective study on CA-BSI in coronavirus disease 2019 ICU showed also the prevalence of *S. epidermidis* [288]. Other prevalence rates have been also reported: 31.37% [289], 31% [290], 28% [291], 18.1% [292], 12.33% [286], 8.3% [293], and 7.7% [261]. The European Center for Disease Prevention and Control reported that *S. epidermidis* caused 23.6% of CA-BSI cases in ICUs [261]. It has also been reported that children are very susceptible to infection associated-methicillin-resistant *S. epidermidis* strains in perinatal units [294]. In addition, in recent years, the situation has become complicated with the capacity of *S. epidermidis* strains to form biofilms on medical devices and their increased resistance to antibiotics, mainly methillin resistance, leading to significantly high mortality and morbidity rates and medical costs [295,296]. It has been reported that more than 70% of *S. epidermidis* strains are resistant to methicillin, which is encoded by *mecA* gene, making the treatment of infections ineffective but above all that this resistance to methicillin can be quickly disseminated to other Gram-positive strains via horizontal gene transfer [297]. *S. epidermidis* strains produce a variety of virulence factors contributing to their pathogenicity (Table 1). However, unlike *S. aureus*, *S. epidermidis* isolates are weakly virulent, do not produce aggressive toxins, and are commonly non-hemolytic [159]. The main virulence factor is its ability to adhere to the surface of medical devices and form a biofilm [298,299]. During accumulation, *S. epidermidis* produces a major component of the biofilm matrix which is PIA [156]. As for *S. aureus*, PIA, encoded by *ica* opeon, is an essential factor in *S. epidermidis* biofilms allowing their adhesion to surfaces [300]. Cherifi et al. [301] found that the *ica* operon was significantly more present in CR-BSI isolates than in commensal isolates. However, François et al. [274] reviewed that the expression of *ica* operon is not essential in the colonization of a surface, and the presence of other virulence genes such as *atlE*, *fbe*, and *embp* are involved in catheter-related infections linked to *S. epidermidis* biofilms [286] The pathogenesis of *S. epidermidis* is regulated by two key systems, the *agr* and the *sar* regulators, which allow the expression or repression of the virulence genes in a coordinated manner during infection [160]. An early study showed that the *agr QS* system played an important role in the long-term development of *S. epidermidis* biofilm during medical device-associated infections [302].

4.8. *Enterococcus* spp.

Enterococci are Gram-positive, non-motile, lactic acid-producing bacteria widely found in the gut microbiota of humans and animals [303]. Enterococci are facultative anaerobes that tolerate a variety of environmental conditions such as extreme pH, salinity, and a wide temperature range (10 to >45 °C) [163]. Enterococci are tenacious microorganisms characterized by increased tolerance to desiccation and starvation, making them resistant to environmental stresses [304]. The emergence of *Enterococcus* strains as HAIs agents [305] can be explained by the following: (i) these bacteria are intrinsically resistant to several classes of antibiotics (cephalosporins, macrolides, clindamycin, and trimethoprim–sulfamethoxazole) and they acquired resistance to ampicillin, ciprofloxacin, high-level aminoglycosides, and vancomycin [306], causing a serious problem for the treatment of these infections given the limited choice of available antibiotics (linezolid, daptomycin, quinupristin/dalfopristin, and vancomycin) [307]; (ii) they have an impressive capacity to acquire new resistance genes due to the plasticity of their genome [308].

Enterococcus spp., particulary *En. faecalis* and *En. faecium*, are the most opportunistic pathogens causing several infections, including medical device-associated infections, UTIs, wound infections, and BSIs [308]. *En. faecalis* is responsible for 80 to 90% of cases of Enterococci-associated NIs, followed by *En. faecium* (5 to 10% of infections) [309,310]. Putta et al. [311] revealed that *En. faecalis* was the most causative agent of CA-UTIs in ICU-patients. In another study, 13.11% of *En. faecium* was isolated from CA-UTIs [312]. Different rates were obtained in other studies: 91% [313], 29% [84], 22.9% [314], 19% [315], and 7.1% [316]. The presence of these pathogens in CL-BSIs was also reported [317,318]. *En. faecalis* was the 5th most frequently isolated bacteria from CA-UTIs and the 3rd from CLA-BSIs, unlike *En. faecium*, which was the 11th and 5th in CA-UTIs and CLA-BSIs, respectively [303]. However, in recent years, the prevalence of infections due to *En. faecium* has increased, overtaking the prevalence of *En. Faecalis*, and this is due to the emergence of antibiotic resistance, especially vancomycin-resistant *En. faecium* (VREfm), which is responsible for the most vancomycin-resistant *Enterococcus* (VRE) infections in the world [319–321], thus leading the WHO to include VREfm in the list of high priority pathogens [321].Of note, glycopeptides resistance in Enterococci is attributed to the acquisition of different clusters of genes (e.g., *vanA*, *vanB*, *vanD*, *vanE*, *vanG*, and *vanL*) which confer resistance [322,323]. In addition to antibiotic resistance, the ability of *Enterococcus* strains to form biofilms is one of the primary factors involved in their virulence and pathogenicity notably on medical devices [324,325]. Several investigations have been conducted on Enterococci virulence factors involved in biofilm formation. Soares et al. [326] found that *En. faecalis* possessed *esp*, *gelE*, and *asa1* genes. Kafil and Mobarez [327] reported the presence of *esp*, *ebpA*, and *ebpB* genes found in high biofilm producers. Similar results were found in the study of Khalil et al. [325]. It has been reported also that the endocarditis and biofilm-associated (Ebp) pilus is involved in biofilm formation on UC leading to CA-UTI [327,328]. However, in presence of urine, Ebp is not capable to initiate *En. faecalis* adhesion on UC. This fact was explained by the release of fibrinogen covering the catheter following an inflammatory response caused by the catheter itself [327]. In another study, the analysis of mutations affecting two proteases, secreted by *En. faecalis* (GelE, SprE), revealed that loss of both factors resulted in decreased CA-UTI and defective biofilm establishment in a murine CA-UTI model, whereas the loss of either had no effect. They revealed also that the high expression of these proteases depends on the *fsr QS* system [329]. Another study reported the importance of Ace and Esp adhesins in the bacterial attachment on the catheter surface, and biofilm accumulation [330].Given that *En. faecalis* is the most identified species in biofilm-associated infections [331,332], the virulence factors cited in Table 1 only concern this bacterial species.

5. Pathogenesis of Venous Catheter Contamination and Catheter-Associated Bloodstream Infections (CA-BSIs)

After insertion of a VC, the surface of the device is immediately covered by a conditioning film composed of organic molecules such as fibronectin and fibrinogen, collagen, elastin, and laminin [27,333]. There are several pathways involved in catheter contamination: colonization of the surface of the catheter tip by skin microorganisms originating from health care worker's hands, contaminated disinfectant, which migrates to the insertion site in the skin pathway of the catheter and along the catheter surface (extraluminal) which is the most common route of infection for short-term catheters (inserted for $\leq$14 days), colonization of the inner surface of the catheter by contaminated infusion product and catheter hub (intraluminal), and finally, by hematogenous seeding (rare route) [17,334]. In contact with blood, microorganisms interact with fibrin to produce an adherent biofilm which will promote bacterial colonization and the spread of these microorganisms [334].

Colonization and biofilm formation on the catheter surface occurs 24 h after device insertion [335]. These pioneer bacteria allow the attachment of other pathogens by providing more diverse adhesion sites. After multiplication, the bacteria produce an extracellular matrix which maintains the biofilm, leading to irreversible adhesion to the surface of the catheter [27]. Once the biofilm is mature, bacteria can disperse, cause catheter associated bloodstream infections (CA-BSIs), and colonize other sites in the body [336].The formation of fibrin sheaths is also observed. Once the catheter is implanted, the fibrinogen, albumin, lipoproteins and coagulation factors, released due to the lesion of the blood vessels, begin to deposit on the surface of the catheter within 24 h forming a fibrin sheath which covers the surface of the catheter within days or even weeks. This fibrin sheath is responsible for the late stage catheter dysfunction, which usually occurs about three months after the catheter placement [333,337].

6. Pathogenesis of Urinary Catheter Colonization and Catheter-Associated Urinary Tract Infections (CA-UTIs)

Transurethral ascension of microorganisms is the most common mechanism for the development of UTIs, which explains the increased risk of infection after catheterization [338]. Bacteria can colonize UCs either by the endoluminal route which involves exogenous flora originating from colonization of the collecting sac or from a breach of the closed system during manipulations of the urinary catheter, or by the exoluminal route which involves the endogenous flora of the urinary meatus and occurs early during catheter placement or later following colonization of the urinary meatus by the digestive flora [6]. After insertion of the catheter, the bacteria overcome first the electrostatic repulsion observed between bacterial cell and catheter surface to allow intimate interactions to occur, then adhere to a conditioning film of urine components and host proteins, such as Tamm–Horsfall protein, magnesium and calcium ions, which form along the catheter surface. It is also reported that the urinary catheter elicits an inflammatory response resulting in the release of the host protein fibrinogen into the lumen of the bladder which will cover the surface of the catheter [330].Pathogens such as *S. aureus* and *Enterococcus faecalis* possess adhesins such as the fibronectin-binding protein A (FnBPA) and the endocarditis and biofilm-associated pilus (EbpA), respectively, which bind to the host fibrinogen in order to disrupt blood clotting, initiate biofilm formation, as well as immune evasion [339,340]. Once irreversibly fixed to the surface of the catheter via adhesins and pili, bacteria begin to change their phenotype, producing exopolysaccharides which protect them and form a biofilm [106,341]. The presence of biofilms promotes the appearance of epithelial lesions due to the proteases and bacterial toxins produced. The uropathogenic bacteria can then ascend to the kidneys, attaching again to the renal epithelium, causing kidney infections. Left untreated, these infections can progress to bacteremia by crossing the tubular epithelial cell barrier into the bloodstream [341].

7. Prevention of CA-BSIs

7.1. Education, Training and Surveillance

The lack of knowledge and skills is one of the main obstacles to medical practice. Indeed, compliance with guidelines for the use of intravascular catheters is very important in order to decrease the incidence of CA-BSIs and their associated health costs. Educating healthcare personnel regarding techniques for using intravascular catheters, the procedures for inserting and maintaining intravascular catheters, periodically assessing their knowledge and ensuring appropriate levels of nursing staff in intensive care units are a first line of prevention [342,343]. In addition, another effective measure to reduce CA-BSIs is to avoid unnecessary catheterization of patients as well as the rapid removal of venous catheters which are no longer necessary, particularly long-term catheters [344].

7.2. Aseptic Techniques

Hand hygiene before handling catheters, disinfecting catheter sites, catheter hubs or injection ports with an appropriate agent before accessing the catheter are essential for the prevention of CA-BSIs [345]. The use of 2% chlorhexidine–alcohol as an antiseptic agent before insertion of a VC and during dressing changes is recommended to prevent the development of CA-BSIs. The incidence of CA-BSIs was shown to be five times lower using 2% chlorhexidine–alcohol solution, compared to 5% polyvidone iodine–alcohol [346]. The catheter tip is also a major source of contamination. For this, its disinfection with appropriate antiseptic or antimicrobial ointments is recommended. Use of a povidone–iodine antiseptic ointment or bacitracin/gramicidin/polymyxin ointment at the exit site after catheter insertion is recommended [335]. The use of sterile gloves, a sterile long-sleeved gown, mask, and large sterile sheath sheet during insertion of a CVC are essential for the prevention of CA-BSIs. A checklist should also be used to improve adherence to procedures at the time of insertion [347]. After insertion of the catheter, the risk of infection should decrease with the use of aseptic techniques. However, insertion and maintenance of VCs by inexperienced personnel could increase the risk of catheter colonization and the development of infection. Having an experienced infusion therapy team in place to insert and maintain catheters decreases CA-BSI levels up to eight times [27].

7.3. Catheter Insertion Site

The catheter insertion site is an important parameter whose choice should be based on both the benefits and risks of the procedure (infection, thrombosis, and mechanical complications). The subclavian site is the ideal insertion site for CVCs, which helps reduce infectious complications [347]. This is probably explained by the fact that the subclavian route has the longest subcutaneous distance between the skin and the entrance to the vessel [348]. In addition, according to previous studies, subclavian catheterization was associated with a lower risk of infectious and thrombotic complications than femoral and jugular catheterization [349–351].

7.4. Catheter Lock Solutions

Another approach that shows promise for the prevention of CA-BSIs is antimicrobial lock therapy. It involves instilling a highly concentrated antimicrobial solution into the lumen of the catheter when not in use [352,353] to remove the blood so that the occlusion and bacterial growth are minimized [5] and also preventing biofilm formation [354]. This technique is useful especially in cases of uncomplicated long-term CA-BSIs caused by pathogens [47]. A variety of antimicrobial agents can be used such as heparin (anti-occlusion) [355,356], vancomycin, gentamicin (antibiotics) [357,358], citrate, ethanol, and taurolidine (antimicrobials) [357,359–361]. Antibiotics are generally used for therapeutic measures once a CA-BSI has been diagnosed, while heparin, citrate, ethanol, and taurolidine are used prophylactically [5]. Furthermore, Kumar et al. [362] demonstrated that using S-nitroso-N-acetyl-l-cysteine ethyl ester (SNACET)is very effective; this is able to generate

nitric oxide with antimicrobial properties as a catheter locking solution. Indeed, a significant reduction of 99% in the adhesion of *S. aureus* and *E. coli* on catheters was observed.

7.5. Dressing

To prevent complications for patients, a dressing is often placed where the integrity of the skin is compromised. Several materials are used as dressing for VCs [362,363]. A gauze dressing is often used when blood seeps from the catheter insertion site. However, their use increases the risk of bacterial contamination and infection [347,364]. Transparent semi-permeable dressings are widely used and allow continuous observation of the skin insertion site and reduce the risk of extrinsic colonization. They should be changed immediately if they become wet, loose, or soiled [347]. The risk of CA-BSI increases more than 3-fold after rupture of the second dressing and more than 12-fold if the final dressing is ruptured [365,366]. The chlorhexidine-impregnated dressing, an innovative strategy, shows promising results in the prevention of infections linked to CVCs, with a reduction in colonization (6.5% versus 13.2%) [367] and infection (1.51/1000 versus 5.87/1000 catheter-days) compared to traditional dressings [368]. Moreover, Puig-Asensio et al. [369] reported that chlorhexidine dressings reduced the risk of CA-BSIs in patients with short-term CVCs, including those with an onco-hematological disease. There is also other evidence that shows that dressings impregnated with chlorhexidine may reduce the risk of CVC-BSI, compared to standard polyurethane dressings, and other types of non-impregnated dressings (gauze and tape dressing) [369–373]. A recent study conducted by Hou et al. [374] found that the chlorhexidine gluconate gel dressings used more effectively reduced the risk of CVC-BSI in patients unlike the chlorhexidine gluconate sponge dressings.

7.6. Antimicrobial Agents Release

Several strategies using coating or impregnating catheters with antibiotics/antimicrobials, peptides, metals, nitric oxide, or other compounds have been developed to prevent biofilm formation and constitute a promising alternative to reduce infection rates and CA-BSIs [375,376]. The use of antimicrobial agents as a coating is the most popular approach due to their ability to target microorganisms in different ways [377]. Inhibition of bacterial adhesion on catheter surfaces could be prevented by releasing the antimicrobial agent [378]. This approach aims to attach the antimicrobial agent to the catheters by adsorption, which will diffuse after exposure to body fluids [379]. This approach allows the release of high doses of the antimicrobial agent without exceeding the toxic threshold, reducing the development of resistance. However, the drawback with this technique is that the release is uncontrolled and lacks long-term properties [380,381].

Catheters coated with chlorhexidine–silver sulfadiazine, minocycline–rifampicin and miconazole and rifampin are the most commonly studied and are associated with a decreased prevalence of catheter colonization and CA-BSIs [382–385]. In fact, it has been reported that these impregnated catheters had the potential to reduce the risk of colonization of these devices and the incidence rates of CA-BSIs per 1000 catheter days [5]. Among catheters based on antimicrobial agent release which have been approved and commercialized, there is ARROWg+ard® (chlorhexidine and silver sulfadiazine coating), Spectrum® (minocycline and rifampin coating) and Chlorag+ard® (chlorhexidine coating) [5]. Other agents are able to reduce bacterial colonization on venous catheter surfaces. Table 2 summarizes most research studies which tested the antimicrobial agent coating/imprenated and surface modifications approaches for the prevention of bacterial colonization and CA-BSI.

Table 2. The effectiveness of different antimicrobial agent coating and surface modification approaches.

Strategy	Agent Used	Approach Used for Coating VC	Microorganism	Reference
Release killing	*Antirhumatic*			
	Auranofin	Auranofin-coated polyurethane catheter.	MRSA	[386]
Release killing	Auranofin	Auranofin-coated polyurethane catheter.	*S. aureus*	[387]
Release killing		*Guanidine derivated*		
	poly(hexamethylene biguanide) hydrochloride–sodium stearate (PHMB–SS)	Coating developed using electrostatic interaction based on polyelectrolyte.	*E. coli* *S. aureus*	[388]
Contact killing		*Antimicrobial peptides*		
	ε-Poly-L-lysine	Electrostatic interaction between cation PL and anion surfactant, 1,4-bis(2-ethylhexyl) sodium sulfosuccinate (AOT).	*E. coli* *S. aureus*	[389]
Contact killing	Poly(methacrylic acid) (PMAA)	Polyurethane surface-initiated atom-transfer radical polymerization (SI-ATRP)	*E. coli* *S. aureus*	[390]
Release killing		*Nitrix oxide*		
	Boron carbon nitride (BCN)	Boron carbon nitride nano-coating using RF magnetron sputtering technique.	*E. coli*	[391]
Release killing	*S*-nitroso-*N*-acetyl-penicillamine (SNAP)	Incorporation of a nitric oxide (NO) donor molecule, *S*-nitroso-*N*-acetyl-penicillamine (SNAP) in a hydrophobic medical grade polymer, Elasteon-E2As and coated with fibronogen.	*E. coli* *S. aureus*	[392]
Release killing	*Metal*			
	Silver nanoparticles (AgNP) Zinc oxide (ZnO)	Incorporation of silver nanoparticles (AgNP) and ZnO nanowires with polyvinylchloride(PVC).	*S. aureus*	[393]
Release killing	Silver	Synthesized novel silver(I) cyanoximates Ag(ACO), Ag(BCO), Ag(CCO), Ag(ECO), Ag(PiCO), Ag(PICO) (yellow and red polymorphs), Ag(BIHCO), Ag(BIMCO), Ag(BOCO), Ag(BTCO), Ag(MCO) and Ag(PiPCO).	*P. aeruginosa* *S. aureus*	[394]

Table 2. *Cont.*

Strategy	Agent Used	Approach Used for Coating VC	Microorganism	Reference
		Quaternary ammonium compounds		
Contact killing	Quaternary ammonium thiol compound (Q8-SH)	Grafting a quaternary ammonium thiol compound (Q8-SH) to a thermoplastic polyurethane containing allyl ether (allyl-TPU) side-chain functionality.	*E. coli* *P. aeruginosa* *S. aureus*	[395]
		Graphene derivated		
Contact killing	Graphene oxide	Immobilization of oxidized graphene nanoplatelets (GNP-M5ox) on the surface of silicone rubber by dip and spray coating.	*S. epidermidis*	[396]
		Other compounds		
Contact killing	poly(dimethylsiloxane) (PDMS)	Hydrophobic hyperbranched coating resin was covalently attached to PDMS.	*E. coli* *P. mirabilis* *S. aureus* *S. epidermidis*	[397]
	Hydrophilic polymer			
Surface modification	Poly(ethylene glycol) PEG	Microcrystalline sulfamethoxazole (SMZ) and trimethoprim (TMP) were immobilized with PEG.	*E. coli* *S. aureus*	[398]
Surface modification	Fluoropolymer	A commercially polyurethane PICC catheter was modified by a three-step lamination process, with thin fluoropolymer layers to yield fluoropolymer–polyurethane–fluoropolymer composite structure before applying the liquid perfluorocarbon (LP)	*S. aureus* *S. epidermidis*	[337]
		Hydrophobic polymer		
Surface modification	Polytetrafluroethylene (PTFE)	SiO_2 nanosphere was coated on PTFE catheter.	*E. coli* *S. aureus*	[399]

7.7. Contact Kill Systems

Contact destruction of bacteria relies on the use of antimicrobials grafted onto the surface of catheters to form a lethal barrier for these pathogens [5]. Indeed, these antimicrobial molecules are mainly cationic or enzymes that bind covalently to the surface of the catheter via hydrophobic polymer chains, and kill these bacteria on contact via membrane interactions.Additionally, this strategy exhibits longer antimicrobial activity and low toxicity [379] and does not output biocides in body fluids [397]. Several compounds such as quaternary ammonium compounds, peptides, graphene derivated (Table 2) have been evaluated as promising contact killing agents. However, the major concern with this strategy is that the bioactive surface can be inactivated when coated with proteins from body fluids [379]. For that, further research studies are needed to improve the strategy.

7.8. Antifouling Approaches

Surface modifications, such as hydrophilic polymeric surface coatings, work also by reducing microbial adhesion to the catheter surface, thereby minimizing infection [375]. Indeed, surface hydration is an important parameter of antifouling coatings due to the water layer formed on the surface of the polymer, which acts as a barrier preventing bacterial adhesion and proteins adsorption [376].The most hydrophilic polymers used in the antifouling approach are poly(ethylene glycol) (PEG) (most commonly used), poly-2-hydroxyethyl methacrylate, poly(2-hydroxypropyl acrylamide), dextran, and zwitterionic polymers [400]. The immobilization of zwitterionic compounds or PEG provides promising results for CR-BSI prevention [398,401]. A recent technology which is the fluoro-passivation of catheters has emerged as an effective approach which consists of coating the catheter with fluoropolymer to increase its biocompatibility and reduce infection [337]. Among the coated catheters commercialized are the AngioDynamics BioFlo PICC catheter (endexo) and the CerebroFlo extraventricular drain catheter (endexo) [337]. Furthermore, another prevention way is the use of materials characterized by low energy, such as hydrophobic polymers (PTFE) [5].A few studies on surface modifications of venous catheters are cited in Table 2. Figure 2 illustrates the main prevention strategies of CA-BSIs.

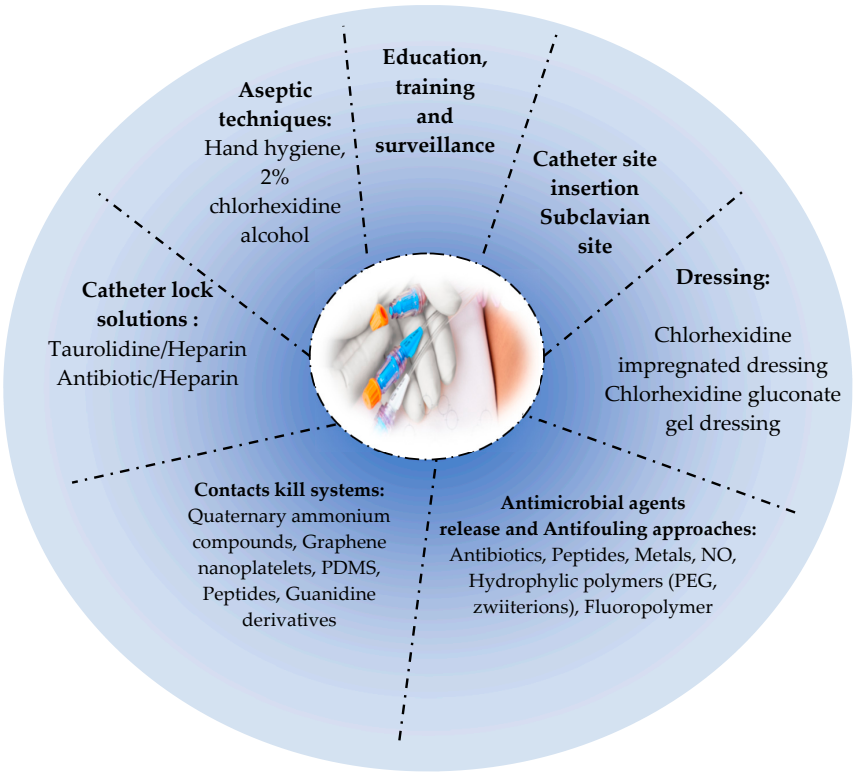


Figure 2. Main prevention strategies of CA-BSIs.

8. Prevention of CA-UTIs

CA-UTI is one of the most common device-related infections in which preventive measures should be taken [402]. These precautions to prevent the transmission of MDR-bacteria must be scrupulously observed in catheterized patients and also limit the uncontrolled use of antibiotics [403].

8.1. Avoidance of Urinary Catheter Use

The main CA-UTI prevention strategy is to avoid or reduce the use of catheters. Overall, UCs are overused and placed for inappropriate indications in 21–50% of catheterized patients [403]. Accepted indications for the use of a catheter are considered the first step in limiting their uses. Among these limitations are the following: urological surgery, monitoring of urine flow in seriously ill patients, management of acute urinary retention and urinary obstruction, or for end-of-life care to improve patient comfort [404]. Limiting the duration of catheterization is also very important. Indeed, when a catheter is placed, it must be removed quickly once it is no longer needed [404]. Healthcare providers should be aware of the existence of the UC. Therefore, catheter remainder interventions that include a verbal/written reminder, a sticker reminder on the patient's chart or an electronic reminder that indicates that a urinary catheter is still in place is a good prevention strategy. Another type of intervention called a "stop order" that requires the clinician (nurse or doctor) to remove the catheter after a period of catheterization or a condition has occurred, unless the catheter remains clinically appropriate, can be followed also in case the reminders are ignored [405]. Institutional policies should also reduce the use of peri-operative catheters by encouraging early removal of post-operative catheters and monitoring bladder volume using ultrasound bladder scanners [404].

8.2. Alternatives to Indwelling UC

Studies have shown decreased UTIs or deaths in patients who used condom catheters. In addition, this type of catheter seems to be less painful and more comfortable than indwelling catheters. Therefore, condom catheters may be an alternative for patients with retained or obstructed bladder. It has also been reported that the use of intermittent catheterization may be beneficial in long-term catheterized patients with neurogenic bladder or after hip surgery has reduced the risk of bacteriuria thereby minimizing the need for an indwelling catheter [403].

8.3. Education and Training

Health care personnel and others who handle urinary catheters should be trained in the procedures for inserting, maintaining, and removing urinary catheters. Education should also be offered on catheter associated urinary tract infections, complications of urinary catheterization, and alternatives to indwelling catheters [406].

8.4. Aseptic Techniques for Insertion and Maintenance of UCs

When indwelling catheterization is required, aseptic catheter insertion and maintenance are recommended to prevent CA-UTIs. For this, UCs must be placed by a qualified healthcare professional [403]. Among these recommendations are the following: hand washing with soap and water should be carried out immediately before and after handling a urinary catheter; the surface of the urethral meatus must also be clean before insertion of the urinary catheter; the catheter must be attached to the patient's thigh to avoid lesions of the urethral meatus; in case of any skin irritation, the catheter should be changed immediately; sterile and closed urine drainage should be used to reduce the risk of infections; finally, irrigation of the bladder with normal saline or a solution containing antibiotics is not recommended, except in cases of obstruction [407].

8.5. Antimicrobial Coatings

Although improved hygiene procedures, replacement of UCs, and the use of prophylactic antibiotics have helped to reduce the incidence of CA-UTIs, it has not been avoided sufficiently. One of the most promising approaches is the use of antimicrobial coatings on UC surfaces to prevent CA-UTIs but more specifically to prevent adhesion, biofilm formation and encrustation of catheters [32,408–411]. Such strategies reduce the viability of pathogens by inhibiting the metabolic pathways necessary for their survival such as inhibiting the synthesis of nucleic acids and proteins involved in cell wall synthesis [410]. However, the development of these devices with antimicrobial surfaces must meet certain requirements, including easy and reproducible production, resistance to mechanical stresses, biocompatible and non-toxic, antimicrobial efficacy for a long time, and finally avoiding the development of resistance [411]. Among the antimicrobial agents used for UC coating are the following: metal (silver, nanoparticles), antibiotics, nitric oxide, antimicrobial peptides, bacteriophages [410]. Table 3 summarizes most research studies which tested the antimicrobial agent coating for the prevention of bacterial colonization and CA-UTIs.

Table 3. The effectiveness of different antimicrobial agent coating and surface modifications approaches used in CA-UTIs prevention.

Strategy	Composite Used	Approach Used for Coating VC	Tested Microorganism	Reference
Release killing	*Metal*			
	Silver (Ag)	Silver–polytetrafluoroethylene (Ag-PTFE) nanocomposite coated UCs.	*E. coli* *P. mirabilis*	[409]
Release killing	copper ions (Cu)	Copper ions (Cu) and a polyphenol tannic acid (TA) were coated on urinary catheters (TA-Cu coated urinary catheters) using using one-step coordination method.	*E. coli* *P. mirabilis* *S. aureus*	[412]
Release killing	*Nanoparticles*			
	Silver (Ag-NPs)	Silver nanoparticles–polydopamine (AgNPs-PDA) coated catheters were designed.	*E. coli*	[413]
Release killing	Copper oxide (CuO-NPs)	Zn-doped CuO-NPs were coated on urinary catheters by sonochemical method.	*E. coli* *P. mirabilis* *S. aureus*	[414]
Release killing	Zinc oxide (ZnO NPs)	Zinc oxide nanoparticles (ZnO NPs) were decorated with amylase (biofilm matrix-degrading enzyme) by sonochemical method.	*E. coli* *S. aureus*	[415]
Release killing	*Antibiotics*			
	Chlorhexidine	Chlorhexidine-loaded poly(ε-caprolactone) nanospheres (CHX-NS) spray-adhered on urinary catheters.	*E. coli* *S. aureus*	[416]
Release killing	Chlorhexidine Triclosan	Chlorhexidine/Triclosan impregnated on silicone catheters.	*E. coli* *K. pneumoniae* *P. mirabilis* *E. feacalis*	[417]
Release killing	Sparfloxacin	Sparfloxacin-coated latex catheters using two immobilization methods.	*E. coli* *S. aureus*	[418]
Contact killing	*Antimicrobial peptides*			
	E6 (RRWRIVVIRVRRC)	A cysteine labeled peptide E6-coated polyurethane catheter was designed by covalent immobilization.	*P. aeruginosa* *S. aureus*	[419]
Contact killing	Chain201D (KWIVWRWRFKR) (from crowberry endophytes)	Chain201D coated on silicone surface model was designed by covalent immobilization.	*E. coli* *S. aureus*	[420]

Table 3. *Cont.*

Strategy	Composite Used	Approach Used for Coating VC	Tested Microorganism	Reference
Contact killing	Cys Lasio-III	Cys Lasio-III was immobilized on a commercial silicone catheter via a combination of AGE brush and PEG based chemical coupling.	*E. coli* *P. aeruginosa* *S. aureus* *En. faecalis*	[421]
	Nitric oxide			
Release killing	Nitrix oxide (NO)	NO-impregnated catheters were designed.	*E. coli*	[422]
	Bacteriophages			
Contact killing	The anti-*Pseudomonas* phage cocktail: ΦPaer4, ΦPaer14, M4, 109, ΦE2005-A, and ΦE2005-C The anti-*Proteus* phage cocktail: ΦPmir1, ΦPmir32, ΦPmir34, and ΦPmir37	Hydrogel-coated catheters were pretreated with phages.	*P. mirabilis* *P. aeruginosa*	[423]
Contact killing	The phage cocktail (podovirus vB_PmiP_5460 and myovirus vB_PmiM_5461)	Urinary catheters treated with phage cocktail were performed.	*P. mirabilis*	[33]
	Hydrophilic polymer			
Surface modification	Poly(N,N-dimethylacrylamide) (PDMAA)	The poly(N,N-dimethylacrylamide) (PDMAA) hydrogel coated on polyurethane ureteral stents.	*E. coli*	[424]
Surface modification	Poly(N,N-dimethylacrylamide) (PDMAA)	The hydrogel coating layer was formed using UV-crosslinking and swell-peeling methods.	*S. aureus*	[425]
Surface modification	Polydopamine/poly(N,N-dimethylacrylamide)	Polydopamine/poly(N,N-dimethylacrylamide)-coated silicone catheters (PDA/uhPDMA) using dip coating approach.	*P. aeruginosa*	[426]
Surface modification	Polyethylene glycol PEG	Silver-polyethylene glycol (mPEG-$DOPA_3$) coated urinary catheters by cross-linking approach.	*E.coli*	[427]
Surface modification	Sulfobetaine methacrylate (SBMA)	Sulfobetaine methacrylate (SBMA) was grafted on silicone catheters using enzymatic approach.	*P. aeruginosa* *S. aureus*	[428]
Surface modification	Polytetrafluoroethylene PTFE	Silver-polytetrafluoroethylene (Ag-PTFE) nanocomposite grafted catheters were developed via a facile wet chemistry method.	*E. coli* *S. aureus*	[429]

Table 3. *Cont.*

Strategy	Composite Used	Approach Used for Coating VC	Tested Microorganism	Reference
	Enzymes			
Surface modification	Acylase	The immobilization of the enzyme on urinary catheters was done by layer-by-layer deposition technique.	*P. aeruginosa*	[430]
Surface modification	α-chymotrypsin (α-CT)	α-chymotrypsin (α-CT) covalently immobilized on low-density polyethylene surfaces (LDPE-α-CT).	*E. coli*	[431]
Surface modification	Glycoside hydrolases (Ghs)	PslGh modified surfaces using amine functionalization (APTMS) and glutaraldehyde (GDA)7 Linking.	*P. aeruginosa*	[432]
Surface modification	Cellobiose deshydrogenase (CDH)	CDH was covalently grafted onto plasma-activated urinary polydimethylsiloxane (PDMS) catheter surfaces.	*S. aureus*	[433]

Metals or composite nanoparticles represent suitable alternatives for CA-UTI prevention and biofilm-related infections [434]. Several studies [409,435–439] have reported that UCs coated with thin-films of silver alloy could reduce bacterial adhesion but also the incidence of asymptomatic bacteriuria and CA-UTI. Other metals have been tested as coatings such as copper (Cu) [412].

Recently, novel advance, namely nanoparticles, constitutes a promising approach in biomedical devices [47]. Several studies reported the efficiency of a silver nanoparticle coating method in colonization prevention of several pathogens such as *E. coli*, *P. mirabilis*, *P. aeruginosa*, *Stapholococcus* spp., and *Enterococcus* spp. [413,429,440–443]. Throughout the years, other nanoparticles have been studied as coating, including green–silver nanoparticles [444,445], gold nanoparticles [446], copper nanoparticles [447] and zinc-doped(Zn) copper oxide (CuO) nanoparticles [414,448].

Antibiotics have been extensively studied over the years and despite the emergence of MDR-pathogenic bacteria, several studies have shown the effectiveness of many antibiotics on infections caused by Gram-negative and Gram-positive bacteria [449]. Moreover, antibiotics are often used for CA-UTI treatment [378]. However, due to their high cost and conflicting results between in vitro studies and clinical trials, their use is questionable [377].Antibiotics that have been commonly studied are such as nitrofurazone. Nitrofurazone-coated UCs was tested against several pathogen biofilms (*E. coli*, *P. aeruginosa*, *S. epidermidis*, *En. faecalis*) with promising results [450–452]. However, their carcinogenic potential in animal models induced their removed from the market and prohibition by the FDA [410]. Other antibiotics such as gentamicin [453], chlorhexidine [454], ciprofloxacin [455], norfloxacin [456], triclosan [457], and sparfloxacin [418] have been tested as a coating agent. Although giving promising results, the antibiotic-based approach favors the apparition of bacterial resistance for long-term catheters (e.g., triclosan), leading to more serious infections [434,458].

For that, the antimicrobial peptides (AMPs) are considered as the most promising strategies to conventional antibiotics [420].AMPs are host defense peptides widely used in the treatment of biofilms associated with several clinical pathogens and kill them by membrane permeabilization [459,460]. Recently, these peptides (e.g., RK1, RK2, CWR11, Bmap-28, E6, Chain 201D) were tested as coating agents for UCs to prevent biofilm formation and CA-UTIs [419,461–463].

In other studies, nitric oxide (NO), a natural gas molecule, with a short half-life, has been demonstrated as being able to protect the host against several pathogens [464]. Its mechanism of action is that it binds covalently to DNA, proteins, and lipids to inhibit or kill the pathogen [377]. The approach based on NO appears to be a promising alternative to combat bacterial infections and the formation of biofilms [465,466].

Bacteriphages have also been suggested as a new strategy to combat bacterial biofilms. They specifically infect bacteria and disrupt their metabolism to self-replicate and then, kill them [128,467]. In addition to their specificity and self replication as advantages, they are able to degrade biofilm matrix and prevent resistance development, while the treatment is improved when phages cocktail is used [378,410,468]. Phage therapy has been used to treat wide bacterial infections with little or no side effects constituting a promising technology in clinical application [469]. Until now, several phage-based coating UCs have been developed and tested for uropathogens including *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, and *Staphylococcus* spp. [468,470–473].

8.6. Antifouling Approaches

Antifouling approaches can also prevent bacterial adhesion on UCs and biofilm formation by repelling them without harming them [377]. The principle of these strategies is the acquisition of anti-adhesive properties by physicochemical modifications of catheter surfaces in order to prevent bacterial adhesion in addition to a good antibacterial activity and low toxicity [376,434]. Moreover, this approach provides the advantage of low risk of drug resistance emergence [474]. Additionally, the hydration layer increases patient com-

fort due to low friction during UC placement [434]. There are wide antifouling approaches, especially hydrogels which are the most popular due to their hydrophilic structure which reduces bacterial growth [475]. Poly(tetrafluoroethylene) (PTFE), poly(ethylene glycol) (PEG), polyzwitterion, and enzyme coating were also studied [377] to prevent the development of biofilms on UC surfaces and CA-UTI prevention. These polymers repel foulants due to the formation of a hydration layer on the surface [449]. Among the hydrophilic coatings, some of them have been already commercialized including HydroPlus™ from Boston Scientific, AQ® from Cook Urological, heparin-based coating Endo-Sof™ Radiance™ from Cook Urological, and SL-6 from Applied Medical [434].

Hydrogels are hydrophilic polymers widely studied as coatings due to their excellent hydropilicity, high hydration, and porous structures [476]. Several studies reported the efficiency of this approach [477–479]. However, it was reported that this approach caused the encrustation of catheters which is contradictory to other results [480]. Further studies are needed to provide more information about long-term prevention of CA-UTIs and biofilm formation and validate their effectiveness.

Another polymer used for coating is poly(tetrafluoroethylene) (PTFE), called also teflon which is characterized by high non-stick properties and resistance to bacterial adhesion, making it an excellent option for biofilm prevention [378,409]. The teflon-coated catheters are commercially available from Bard Medical [377]. Various early studies have been also conducted for the same purpose [429,481,482].

Similary, polyethylene glycol (PEG) possesses nonimmunogenic, nonantigenic, and protein repellent properties thus appearing to be a good antifouling agent [377].

Polyzwitterions that contain both cationic and anionic ions constitute also promising antifouling agents for the coating of UCs due to their superhydrophilicity [483]. Researchers designed different polyzwitterion silicone catheter surfaces and studied their effectiveness including sulfobetaine methacrylate (SBMA) [428], copolymer-coated Ti6Al4V (Ti6Al4V@DMA-MPC) [484], polysulfobetaine (PSB) [485], poly(sulfobetaine methacrylate) (pSBMA), and poly(carboxybetaine methacrylate) (pCBMA) [486].

In the recent past, the effectiveness of the enzymes was evaluated toward bacterial adhesion [377]. Furthermore, the enzymes are natural, safe, and non-toxic to other than their target cells which is an advantage. They were recently studied in the UC-coatings field [449,487]. Among the enzymes already tested are the following: acylase, cellobiase dehydrogenase, α-chymotrypsin, and glycoside hydrolases [377]. Table 3 summarizes most of the studies showing the effectiveness of different antifouling approaches. Figure 3 illustrates the main prevention strategies of CA-UTIs.

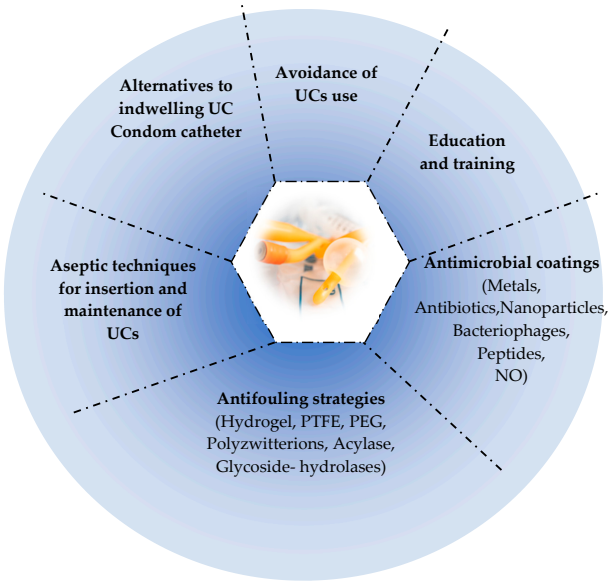


Figure 3. Main prevention strategies of CA-UTIs.

9. Conclusions

Medical device-associated biofilm infections, mainly catheter associated bloodstream infections and catheter associated urinary tract infections, which are the most common infections in healthcare, constitute a real problem in hospitals. In addition to the global emergence of multidrug resistance, the biofilm formation on these devices, especially the presence of persistent cells, makes these infections worse, causing the recalcitrance of infections and therapeutic failure, thereby increasing the rate of morbidity, mortality, healthcare cost, and length of hospitalization.

However, the risk of these infections could be reduced by respecting the prevention guidelines, including educating healthcare personnel, hygiene, limiting use, choice of catheter insertion site, and the antimicrobial lock therapy. In recent years, novel and effective advanced strategies have been developed as contact kill systems—antimicrobial-coated catheters with metals, nanoparticles, phages, antibiotics, antimicrobial peptides and other compounds—and have helped to reduce bacterial adhesion, biofilm formation, catheter encrustation, cytotoxicity, and complications for patients. Antifouling approaches also constitute promising alternatives to prevent medical device associated infections. Despite all the in vitro and in vivo studies that have been conducted in this area, no ideal strategy has been found until now due to the divergence of the results obtained. Further, additional research studies, notably clinical trials, are still needed to develop biocompatible strategies and fully validate their efficiency in order to prevent and fight medical device-associated biofilm infections, especially for long-term catheters.

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Abstract: A total of 219 bacterial strains were isolated from 33 medical devices (Intravenous catheter, urinary catheter and intra-abdominal drain) collected from patients hospitalized at least 48 h in different departments at two hospitals and a private healthcare unit (Bejaia, Algeria). The relevant isolates were *Staphylococcus* spp., *E. coli*, *K. pneumoniae*, *En. faecalis* and *Acinetobacter* spp.. The majority of strains were strong biofilm producers and harbored several virulence genes (*fim*H, *mrk*D, *omp*A, *ica*A, *ica*D, *esp* and *asa*1) involved in the adhesion and biofilm formation. *K. pneumonia*, *A. baumannii*, coagulase negative *Staphylococcus* and *En. faecalis* strains exhibited high levels of antibiotic resistance. A total of 46.12% of the strains were β-hemolytic, the majority of them were moderately hydrophobic (43.38%) and 57.5% of the strains were autoaggregatives (10-20% rates); with a variable co-aggregation rates (1.26-70.48%). In the second part of this study, the efficacy of the peptide SAAP-148 and halicin on four selected Gram negative bacteria colonizing catheters was tested. The bactericidal activity of SAAP-148 and halicin was reduced in urine and plasma as compared to buffered saline. In a dose-dependent manner, both agents reduced bacterial counts in 24 h preformed biofilms on silicone elastomer discs and eliminated persisters, with halicin being less effective than SAAP-148. Importantly, SAAP-148 and halicin acted synergistically on *E. coli* and *K. pneumoniae* biofilms but not on *A. baumannii* biofilms. The peptide, but not halicin, decreased the production of IL-12p40 upon exposure to UV-killed bacteria. Overall, SAAP-148 and halicin constitute a good alternative to combat Gram negative bacteria colonizing catheters.

Keywords: Medical devices, bacterial isolates, biofilm, virulence genes, hemolysis, peptide SAAP-148, halicin, synergy.

Résumé : Un total de 219 souches bactériennes ont été isolées de 33 dispositifs médicaux (cathéter intraveineux, sonde urinaire et drain intra-abdominal) collectés chez des patients hospitalisés au moins 48 h dans différents services de deux hôpitaux et une clinique privée dans la wilaya de Béjaïa, (Algérie). Les isolats obtenus étaient *Staphylococcus* spp., *E. coli*, *K. pneumoniae*, *En. faecalis* et *Acinetobacter* spp.. La majorité des souches étaient fortement productrices de biofilm et possédant plusieurs gènes de virulence (*fim*H, *mrk*D, *fim*H-1, *omp*A, *csu*E, *ica*A, *ica*D, *esp* et *asa*1) impliqués dans l'adhésion et la formation de biofilm. Les souches de *K. pneumoniae* et *A. baumannii*, *Staphylococcus* à coagulase négative et *En. faecalis* présentaient des niveaux élevés d'anbio-résistance. Au total, 46,12 % des souches étaient β-hémolytiques, la majorité d'entre elles étaient modérément hydrophobes (43,38 %) et 57,5% étaient auto-agrégatives (taux de 10-20 %), avec une co-agrégation variable (1,26-70,48%). Dans la deuxième partie de cette étude, l'efficacité du peptide SAAP-148 et de l'halicin sur quatre bactéries Gram négatives sélectionnées colonisant les cathéters a été testée. L'activité bactéricide de SAAP-148 et de l'halicin a été réduite dans l'urine et le plasma par rapport au sérum physiologique tamponné. De manière dose-dépendante, les deux agents ont réduit le nombre de bactéries dans les biofilms de 24 h, préformés sur des disques d'élastomère de silicone, et ont éliminé les bactéries persistantes, l'halicin étant moins efficace que SAAP-148. Il est important de noter que SAAP-148 et l'halicin ont agi en synergie sur les biofilms d'*E. coli* et de *K. pneumoniae*, mais pas sur les biofilms d'*A. baumannii*. Le peptide, mais pas l'halicin, a diminué la production d'IL-12p40 lors de l'exposition à des bactéries tuées par les UV. Dans l'ensemble, SAAP-148 et l'halicin constituent une bonne alternative pour lutter contre les bactéries Gram négatives colonisant les cathéters.

Mots clés : Dispositifs médicaux, isolats bactériens, biofilm, gènes de virulence, hémolyse, peptide SAAP-148, halicin, synergie.

ملخص : تم عزل ما مجموعه 219 سلالة بكتيرية من 33 جهازًا طبيًا (قسطرة وريدية وقسطرة بولية وتصريف داخل البطن) تم جمعها من مرضى تم إدخالهم إلى المستشفى لمدة 48 ساعة على الأقل في أقسام مختلفة في مستشفيين ووحدة رعاية صحية خاصة (بجاية، الجزائر). كانت العزلات ذات الصلة هي *Staphylococcus* spp. و *E. coli* و *K. pneumoniae* و *En. faecalis* و *Acinetobacter* spp.. كانت غالبية السلالات منتجة قوية للأغشية الحيوية وتحتوي على العديد من جينات الضراوة (*fimH* و *mrkD* و *ompA* و *icaA* و *icaD* و *esp* و *asa1*)المشاركة في الالتصاق وتكوين الأغشية الحيوية. أظهرت سلالات *K. pneumoniae* و *A. baumannii* و *Staphylococcus* السلبية للتخثر و *En. faecalis* مستويات عالية من مقاومة المضادات الحيوية. كان إجمالي 46.12٪ من السلالات β- الانحلالية، وكانت غالبيتها كارهة للماء بشكل معتدل (43.38٪) وكانت 57.5٪ من السلالات ذاتية التراكم (معدلات 10-20٪)؛ مع معدلات تراكم مشتركة متغيرة (1.26-70.48٪). في الجزء الثاني من هذه الدراسة، تم اختبار فعالية الببتيد SAAP-148 وهاليسين على أربع قسطرات مستعمرة للبكتيريا سلبية الجرام المختارة. انخفض النشاط القاتل للبكتيريا SAAP-148 وهاليسين في البول والبلازما مقارنة بالمحلول الملحي العازل. بطريقة تعتمد على الجرعة، قلل كلا العاملين من أعداد البكتيريا في الأغشية الحيوية المشكلة مسبقًا على أقراص إلاستومر السيليكون لمدة 24 ساعة وأزالا البكتيريا الباقية، وكان هاليسين أقل فعالية من SAAP-148. ومن المهم أن SAAP-148 و halicin يعملان بشكل تآزري على الأغشية الحيوية لـ *E. coli* و *K. pneumoniae* ولكن ليس على الأغشية الحيوية لـ *A. baumannii*. يعمل الببتيد، ولكن ليس halicin ، على تقليل إنتاج IL-12p40 عند التعرض للبكتيريا المقتولة بالأشعة فوق البنفسجية. بشكل عام، يشكل SAAP-148 و halicin بديلاً جيدًا لمكافحة البكتيريا سالبة الجرام التي تستعمر القسطرة.

الكلمات المفتاحية: الأجهزة الطبية، العزلات البكتيرية، الأغشية الحيوية، جينات الفوعة، انحلال الدم، الببتيد SAAP-148 ، الهاليسين، التآزر.