Ministère de l'Enseignement Supérieur et de la Recherche Scientifique

Université A MIRA-BEJAIA

Faculté des Sciences de la Nature et de la Vie
Département de Microbiologie
Spécialité :Microbiologie fondamentale

Mémoire de fin de Cycle

Pour l'obtention du diplôme de Master

Thème:

Etude de la résistance à la colistine dans les différents environnements

Présenté par :
REDOUANE SIHAM & SLIMANI MERIEM
Soutenu le : 28 Septembre 2021

Devant le jury composé de :

M KECHA.M	**Professeur**	**Président**
Mme TAFOUKT.R	**MCB**	**Encadreur**
Mme LAINCER.F	**MCA**	**Examinateur**

Année universitaire : 2020 / 2021

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Thanks

Above all, we would like to thank the one who protected, helped and supported us, until we can "lead the seed to the fruit" we bow to say:
" Thank God ".
Our warmest and warmest thanks go first to our promoter Mrs TAFOUKT.R for her guidance, advice,guidance and encouragement throughout this work and that without it, thethis work would never be completed.
We would like to warmly thank Mr. KECHA.M . for having accepted tochair the jury as well as Mrs LAINCER.F . For having accepted to examine ourjob.
A big thank you to all those who contributed directly or indirectly to the achievement of this work .
Thanks

-Siham et Meriem-

Dedications

At the will of the god, and A lot of patience and volente I dedicate this work :

In memory of my dear sister who I love toMay God forever welcome her in his vast paradise.

For the love of my life, my dear mother, may God protect you.

To my very dear father I am proud and happy to achieve part of what youhave hoped and expected so much from me. No word, no dedication would knowexpress at its true value, for all the sufferings you have endured. I yousay thank you very much.

To my dear fiancé Adel, who supported and encouraged me.

To my dear sister Sabrina and her little angel Ghaith.

To my brothers Alaa eddine and Dhaia eddine for Their presence by my side.

To all my family, my friends who have supported me a lot and helped me through difficult times.

-Siham -

Dédicace

I dedicate this modest work

TO

My dear parents whom I thank very much for their advice, encouragement and their prayers throughout my course

Dad, mom this work is the fruit of all your sacrifices, I love you May God keep you for us.

My brothers Fouad, Massi, Lakhdar

My only sister Souhila

My dear aunts

My cousins Sara, Hadjer, Hanane, Ryma, Kenza, khokha, celina, aya

My friends: ouahiba, Lydia, Amel, nouwara, Nadira, Samira

My siham partner and his family

All my relatives near and far

-Meriem-

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List of abbreviations

AMR:**A**ntimicrobial**R**esistance

ARGs:**A**ntimicrobial **R**esistance **G**ene**s**

BGN: **B**acilles à **G**ram **N**egative

BLSE: **B**êta-**L**actamase à **S**pectre **E**tendu

BMR : **B**actérie **M**ulti-**R**ésistante

CMI: **C**oncentration **M**inimale **I**nhibitrice

CTX-M: Cefotaxime hydrolyzing capabilities

ESBL:**E**xtended-**S**pectrum **β**-**L**actamases

EUCAST: **Eu**ropean **C**ommittee on **A**ntimicrobial **S**usceptibility **T**esting

LPS :**L** ipo**p**olysaccharide

MCR :**M**obilized**C**olistin **R**esistance proteine

mcr :**M**obilized **C**olistin **R**esistance gene

MDR: **M**ultidrug **R**ésistant

MGCB:**M** cr **G**ene-**C**ontaining **B**acteria

NDM :**N**ew**D**elhi **M**etallo-bêta-lactamase

PCR :**P**olymerase**C**hain**R**eaction

PEtN :**P**hosphoéthanolamine

pmr :**p**olymyxin **R**esistance **O**peron

PMQR :**P**lasmid-**M**ediated **Q**uinolones **R**esistance

Introduction

Introduction

With the introduction of antibiotics in the 20th century and the new easy ways of treating otherwise deadly infection, also the appearance of antibiotic resistance occurred quickly, indeed, With each new antibiotic or antibiotic class that was introduced to the market, resistance towards this new antibiotic or antibiotic class occurred within a short period of time and rapidly increased with the high use of the antibiotics. (Viktoria ,2018).

By defenition, Antibiotic resistance is a way of bacteria to withstand the effect of antibiotic, so that they are not killed or inhibited by the drug, can be acquired by bacteria by two different events: Mutation and Horizontal Gene Transfer (HGT)(Davies, and Davies,2010), Bacteria have acquired multiple mechanisms of resistance including enzymatic inactivation, target site mutation and efflux pumps.(Viktoria ,2018)

Increasing antibiotic resistance in multidrug-resistant (MDR) Gram-negative bacteria (MDR-GNB) presents significant health problems worldwide, since the vital available and effective antibiotics, including; broad-spectrum penicillins, fluoroquinolones, aminoglycosides, and β-lactams, such as; carbapenems, monobactam, and cephalosporins; often fail to fight MDR Gram-negative pathogens as well as the absence of new antibiotics that can defeat these "superbugs". All of these has prompted the reconsideration of old drugs such as polymyxins that were reckoned too toxic for clinical use.(El-Sayed Ahmed and al.,2020)

The polymyxins act on the cell wall of Gram-negative bacteria by way of three known mechanisms of action. Polymyxins are cationic molecules that electrostatically disrupt bacterial surface membranes by displacing Ca^{2+} and Mg^{2+} ions that stabilize lipopolysaccharide (LPS) molecules. Polymyxins are surface-active amphipathic agents containing both lipophilic and lipophobic groups. They penetrate into cell membranes and interact with phospholipids in the membranes, leading to perme- ability changes that quickly disrupt cell membranes leading to cell death. Polymyxins also bind to the lipid A portion of endotoxin or LPS molecules and, in animal studies, block many of the biologic effects of endotoxins..(Chen and Kaye 2009)

Colistin is a polypeptide of the group E polymyxin family. Was obtained in 1947 in japan from a soil bacteria *Paenibacillus Polymyxa subsp.colistinus*. It is a complex mixture of at least 30 compounds, the two major ones are colistin A (polymyxin E1) and colistin B (polymyxin E2). Colistin A and colistin B differ by a fatty acid chain in the terminal

position (Frasca , 2008). it works by binding to lipopolysaccharides (LPS), a component of outer membrane, only present in Gram-negative bacilli. His mechanism of action, not fully elucidated, can be explained by three modes distinct and concomitant: lysis of bacterial membranes (main mode), contact "vesicle-vesicle", and formation of free radicals. These three mechanisms result in the death of the bacteria . (Dortet ,.2016)

The emergence of resistance to colistin has been emerging in recent years and appears to be strongly linked to excessive colistin use and high doses non-optimal favoring selection pressure (Mezghani Maalej ,2012;Hanulík ,2013).The mechanisms underlying polymyxins resistance in Gram Negative Bacteria (GNB) are complex and not completely understood until now. Generally, GNB can develop resistance to polymyxins through intrinsic, mutation or adaptation mechanisms, besides the horizontally acquired resistance mediated via the *mcr-1* gene and its variants (El-Sayed Ahmed and al.,2020).

It is largely recognized that the environment plays a huge role in disseminating clinically relevant antimicrobial resistance genes (ARGs). Experts agree that the control of ARGs' spread cannot be achieved without tackling this problem from the environment. It is established that antimicrobial selection pressure in the environment results due to discharges from humans and animals (containing un-metabolized antimicrobials in faeces/urine into sewage), hospitals, industries, and run-offs from refuse dumps (often contains anthropogenic wastes) and farmlands especially where biocides are used (Anyanwu and al., 2020).

The hospital environment often contains numerous antibiotics which perhaps stimulate colistin resistance. The most worrisome situation about colistin resistance is the presence of other antimicrobial agents (including disinfectants and metals) in an environment, could stimulate colistin selection pressure, and mcrgene is often co-located with multiple AMR (most troubling being carbapenemases, extended-spectrum β-lactamases [ESBL], and plasmid-mediated quinolone resistance [PMQR] encoding genes) and virulence genes on highly promiscuous plasmids. There have been extensive discourse on acquired resistance and virulence genes, as well as the drivers (plasmids, integrons, transposons, insertion sequences, etc.) facilitating the horizontal/lateral spread (by conjugation, transformation or transduction) of these genes among bacterial population (Anyanwu and al., 2020).

In this context, the objective of this work was on the one hand to study the prevalence and distribution of resistance to colistin in the different environments (water, soil, animals) worldwide, and on the other hand to determine the phenotypic and genotypic methods of d detection resistance to colistin.

PART I

Emergence of colistinresistance

I. Mecanism of action of Colistin

Polymyxins, a structurally distinct class of nonribosomal, cyclic oligopeptides antimicrobials, include five chemically distinguished compounds (polymyxins A, B, C, D, and E) of which polymyxin B and colistin (polymyxin E) (Figure 1) are the only two polymyxins currently available on the market variants (El-Sayed Ahmed and al., 2020). The polymyxins act on the cell wall of gram-negative bacteria by way of three known mechanisms of action. Polymyxins are cationic molecules that electrostatically disrupt bacterial surface membranes by displacing $Ca^{2+}$and Mg^{2+} ions that stabilize lipopolysaccharide (LPS) molecules. Polymyxins are surface-active amphipathic agents containing both lipophilic and lipophobic groups. They penetrate into cell membranes and interact with phospholipids in the membranes, leading to permeability changes that quickly disrupt cell membranes leading to cell death. Polymyxins also bind to the lipid A portion of endotoxin or LPS molecules and, in animal studies, block many of the biologic effects of endotoxins (Figure 2)..(chen L.F and Kaye D ,2009)

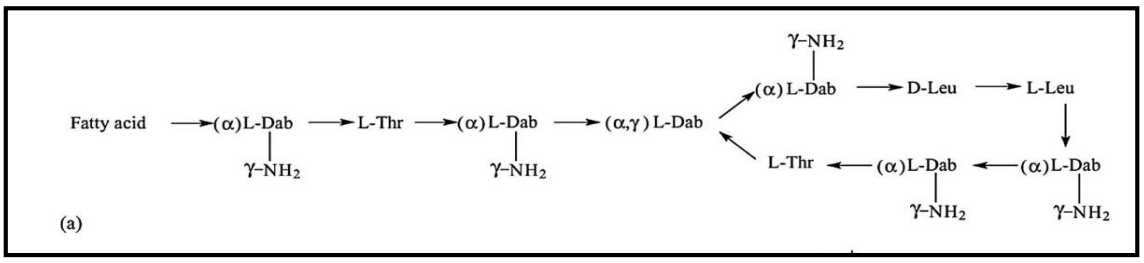


Figure 1 : Chemical structure of colistin (El-Sayed Ahmed and al., 2020)

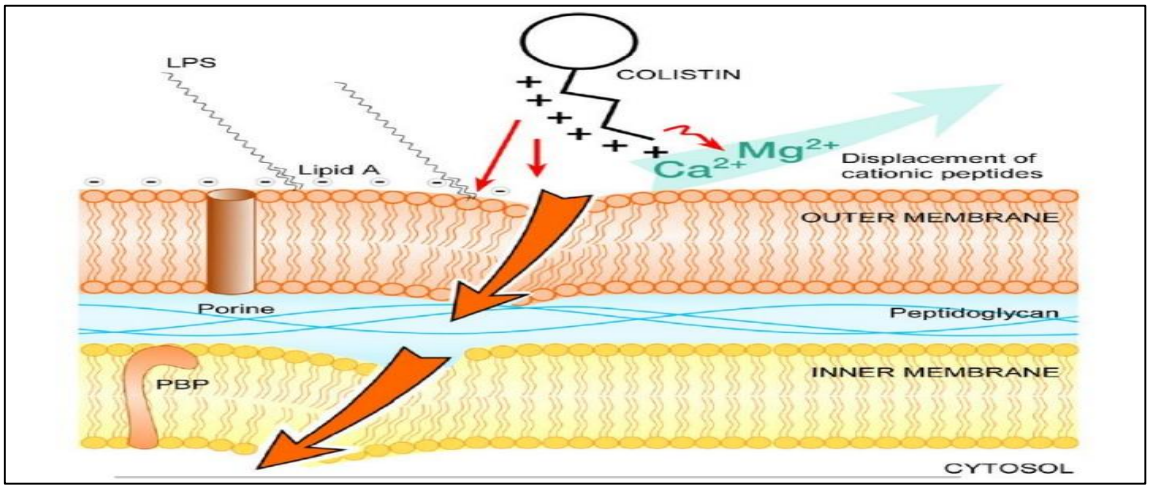


Figure2 : Mecanism of action of colistin on the membrane of gram-negative bacteria (Anne-Gaelle .,2018)

II. Application

Despite its potential nephrotoxicity and neurotoxicity, colistin is still used for treatment as an ultimate line of defense against critical infections caused by MDR pathogens (carbapenemase-producing Enterobacteriaceae) (Xu et al., 2018). His mechanism of action, not fully elucidated, can be explained by three modes distinct and concomitant: lysis of bacterial membranes (main mode), contact "vesicle-vesicle", and formation of free radicals. These three mechanisms result in the death of the bacteria .(Dortet and al,2016).

III.Mecanisms of resistance

III.1.Chromosomic resistance

In Resistance to colistin can be explained by a variety of mechanisms. Until 2015, it was thought to be acquired solely via chromosomal point mutations. As LPS is the target of colistin, any change in it can change the behavior of colistin. *Salmonella* and *E. coli* are able to modify LPS by changing lipid A through the biosynthesis of 4-amino-4-deoxy-L-arabinose (L-Ara4N) and/or phosphoethanolaminePEtN .Its biosynthesis is associated with chromosomal mediated resistance, dependent on two-component response regulators and sensor kinase systems: PmrA/PmrB and PhoP/PhoQ , The first system PmrA/PmrB also controls the pmr HIJKLM operon, which promotes the synthesis of N4 aminoarabinosis, which in turn, when bonding chemically the fractions of lipid A, changes the negative charge of the cell membrane by neutralizing the negatively charged phospholipids. This resistance mechanism is exhibited by *Pseudomonas aeruginosa* .(Andrade and al,2020)

III.2. Plasmidic resistance

In Novembre 2015, a transferable plasmid gene confering resistance to colistin a has been described in *E.coli* and *K.pneumoniae* in China. This is the *mcr1* gene, encoding a phosphoethanolamine transferase, which adds a PEtN group on lipid A. This *mcr1* gene was then described in Enterobacteria of various species (*Enterobacter spp, Salmonella spp, Shigella)* isolated from humans, animals, food and in the environment. The discovery of another *mcr2* resistance plasmid gene in *E.coli* followed in June 2016 in Belgium, (Xavier and al ,2016) ,Since a few other mcr genes including, mcr-3, mcr-4, mcr-5, mcr-6, mcr-7, mcr-8, mcr-9 and mcr 10 have been described (Table I).

All of the MCR proteins are phosphoethanolamine (PEA) transferases. Of note, the discovery of *mcr-9* in colistin-susceptible strains suggests that strains carrying *mcr* genes may not exhibit colistin resistance phenotype due to low-level gene expression (Wang et al., 2020).

Table I: Characteristics of the *mcr* resistance genes described.

Gene	Species	Host	Contry	Year	Reference	Incapability group of plasmids
***mcr* 1**	*E .coli*	Porc	China	2015	(Liu and al,2016)	SHP45
***mcr* 2**	*E. coli*	Porc and bovine	Belgium	2016	(Xavier and al ,2016)	Incx4
***mcr* 3**	*E.coli*	Flies (muscadomestica) and pond water	Bangladesh	2018	(Sobur and al 2019)	/
***mcr* 4**	*S. entirica Serovar Typhimurium*	Pors and human	Italy	2015-2016	(Carattoli and al,2017)	R3445
***mcr* 5**	*S.entirica*	Chicken and food	Germany	2011-2013	(Borowiak and al,2017)	Tb3
***mcr* 6**	*Maraxallaspp*	Porc	United Kingdom	2014-2015	(Abuoun and al ,2017)	/
***mcr* 7**	*Aeromonasspp*	Chiken	China	2010-2015	(Yang and al ,2018)	/

***mcr* 8**	*K .pneumoniae*	Porc	China	2015-2017	(Wang and al ,2018)	IncFII
***mcr* 9**	*Enterobactercloacae* *E .coli* *Klebsiellaoxytoca* *Cytrobacter freundii*	Horse	Sweden	2017-2018	(Borjesson and al, 2019)	IncHi2 And IncHI2A

IV. Colistin resistance in Africa

A systematic review, made by Olowo-okere and Yacouba about molecular mechanisms of colistin resistance in Africa, has shown that, on a total of 36 studies conducted in seven countries namely Tunisia (n=7), South Africa (n=6), São Tomé and Príncipe (n=1), Nigeria (n=2), Libya (n=1), Egypt (n=8), and Algeria (n=11). The studies documented colistin resistance mechanisms in bacterial isolates obtained from humans (21; 58.3%), animals (10; 27.8%) and environmental samples (3; 8.3%). In the included studies, colistin resistance mechanism was described in mainly three bacterial species comprising *A. baumannii*, *E. coli* and *K. pneumoniae*. Colistin resistance was most frequently observed in *E. coli* isolated from human clinical samples. Both chromosomal and plasmid mediated mechanisms were reported, with plasmid-mediated colistin resistance mechanism (26; 72.2%) most frequently reported (Figure3). Overall, the studies described colistin resistance in 904 bacterial isolates, 188 (20.79%) of which harbored various *mcr* genes. Of the 188 *mcr* genes detected, *mcr-1* was the most prevalent. Among the 188 isolates in which the presence of *mcr* genes has been reported, 88 (46.80%) reported the types of plasmid. The *mcr* genes were mostly harbored on an IncHI2 plasmid (n=50; 56.82%), followed by IncI2 (n=19; 21.59%) (Olowo-okere and Yacouba, 2020).

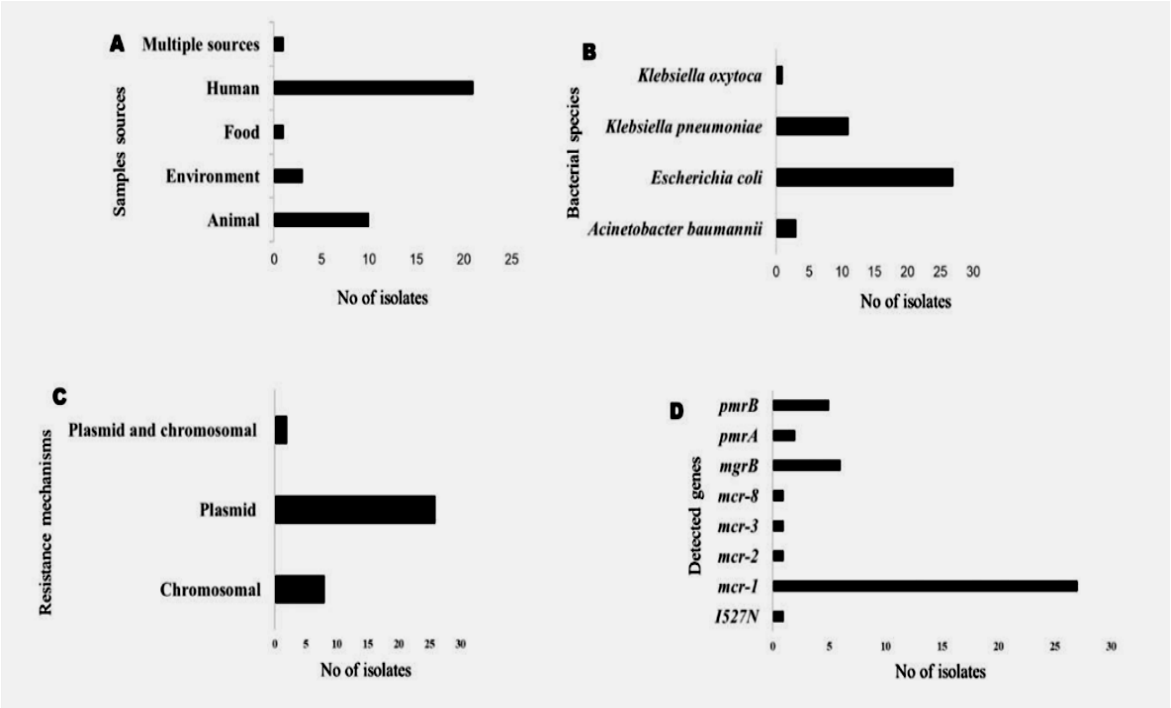


Figure 3 : Characteristics of colistin resistance in Africa (Olowo-okere and Yacouba, 2020).

V . Genetic diversity of mcr gens

The emergence and spread of plasmid-mediated movable colistin (COL) resistance (mcr) genes jeopardize the efficacy of COL considered a last resort drugs for treating deadly infections caused by multi- and extensively drug-resistant Gram-negative bacilli (GNB) (Anyanwu and al,2019).

In November 2015, the first plasmid-located transferable mechanism of resistance to colistin was identified in a porcine isolate of *E.coli* in China. During the following months and years the *mcr-1* (after "mobilized colistin resistance") gene was identified by PCR in *E. coli* isolates from cattle, humans, piglets, poultry in several countries in different continents and in several other species of the *Enterobacteriaceae* family, including *Salmonella (S.) enterica* and *K. pneumonia* (Damien and al., 2019).

Indeed, this heralded the emergence of pandrug-resistant bacteria (superbugs). Since, after the discovery of *mcr-1* gene, eight other *mcr* gene types with their very many variants have been detected in isolates from humans, animals, and environment in six of the seven continents (Anyanwu and al, 2019).

The *mcr-2* plasmid resistance gene (1617 bp) was discovered in strains of *E. coli* isolated in pork and beef in Belgium (Xavier and al, 2016). It is located on a mobile genetic element of the family IS1595 (IS1595 insertion sequences on each side of the *mcr-2* gene and the presence of a transposase type ISXO2-like). This mobile element is carried by a plasmid of the IncX4 type (Xavier and al, 2016). MCR-2 protein (538 aa) shares 80.7% amino acid identity with MCR-1. The progenitor of the gene encoding MCR-2 is most likely *Moraxella pluranimalium* because the mobile genetic element IS1595 has been identified in *M.pluranimalium* and phylogenetic analysis of MCR-2 revealed strong homology (99% identity protein) with a phosphoethanolamine transferase from *M. pluranimalium* (Poirel and al ,2017).

The *mcr-3* plasmid resistance gene (1626 bp) was discovered in strains of *E. coli* isolated from pigs in China. It is carried by an IncHI2 type plasmid carrying a TnAs2 transposon. MCR-3 protein (541 aa) shares 32.5% and 31.7% amino acid identity with MCR-1 and MCR-2, respectively. The progenitor of the gene encoding MCR-3 is thought to be a bacterial species of the genus *Aeromonas* because the TnAs2 transposon has only been identified in *Aeromonas salmonicida* and the analysis phylogenetics of MCR-3 revealed strong homology (94.1-94.8% amino acid identity) with. The phosphoethanolamine transferases from *Aeromonas spp*. (Yin and al ,2017).

The *mcr-4* plasmid resistance gene (1626 bp) was discovered in a strain of *S. enteric serovar Typhimurium* isolated from pigs in Italy and from *E. coli* strains isolated from pigs in Spain and Belgium. It is carried by a ColE10-type plasmid which can be mobilized using a "helper" conjugative plasmid (Carattoli and al, 2017). The MCR-4 protein (541 aa) shares 34%, 35% and 49% of identities in amino acids with MCR-1, MCR-2 and MCR-3, respectively. The progenitor of the gene encoding MCR-4 would be *Shewanella frigidimarina* because the ISKpn6 insertion sequence located upstream of the mcr-4 gene shows 99% nucleotide identity with an insertion sequence identified in *S. frigidimarina* and analysis phylogenetics of MCR-4 revealed strong homology (82-99% protein identity) with phosphoethanolaminetransferases from *Shewanella spp*.(Jayol ,2017).

The *mcr-5* plasmid resistance gene (1644 bp) was discovered in a strain of *S. enteric serovar Paratyphi* B isolated from poultry and food in Germany. It probably does started from an operon which codes for the MCR-5 protein but also for a ChrB protein involved in the chromate resistance and for two fragments of transport proteins from the major

superfamily offacilitators (MFS). The operon containing the *mcr-5* gene is located on a transposon of the Tn3 family carriedby a ColE-type plasmid. MCR-5 protein (547 aa) shares 36.1%, 35.3%, 34.7% and 33.7% amino acid identities with MCR-1, MCR-2, MCR-3 and MCR-4, respectively (Figure 4)(Borowiak and al ,2017).

The *mcr-6* (previously known as *mcr-2.2*) has been discovered in *Moraxella spp.* isolated from pigs in Great Britain. Besides, only one variant of *mcr-6* (*mcr-6.1*) has been identified until now (El-Sayad Ahmed and al, 2019).

The *mcr-7* has been identified in *K. pneumoniae* isolated from chickens in China and also one variant of *mcr-7* (*mcr-7.1*) has been identified until now (El-Sayad Ahmed and al, 2019).

The *mcr-8* was found in NDM-producing *K. pneumoniae* isolated from both pigs and humans in China. Four variants of *mcr-8* were identified, including; *mcr-8.1* to *mcr-8.4.*

Carroll et al. have identified the novel mcr homologue, *mcr-9*, which was isolated from *S. Typhimurium* strain HUM_TYPH_- WA_10_R9_3274. Investigations on the genetic environment of *mcr-9* gene revealed that the amino acid sequence of *mcr-9* most closely resembled those of *mcr-3* and *mcr-7*. Mcr-3.17 has the highest-scoring *mcr* allele, which shares 64.5% amino acid sequence identity with *mcr-9* and 99.5% coverage. Besides, two variants of *mcr-9* have been identified until now, namely; *mcr-9.1* and *mcr-9.2* (El-Sayad Ahmed and al, 2019).

In recent years, a new *mcr* gene has been discovered, *mcr-10*, on an IncFIA plasmid of an *Enterobacter roggenkampii* clinical strain. *mcr-10* has the highest nucleotide identity (79.69%) with *mcr-9* and encodes MCR-10 with 82.93% amino acids identical to MCR-9. mcr-10 confers 4-fold increase in colistin MIC (from 1 to 4 mg/L) when cloned into a colistin-susceptible *E. roggenkampii* strain. By screening GenBank, *mcr-10* was found in various *Enterobacteriaceae* species of countries in four continents, suggesting that this gene has widely spread (Wang and al, 2020).

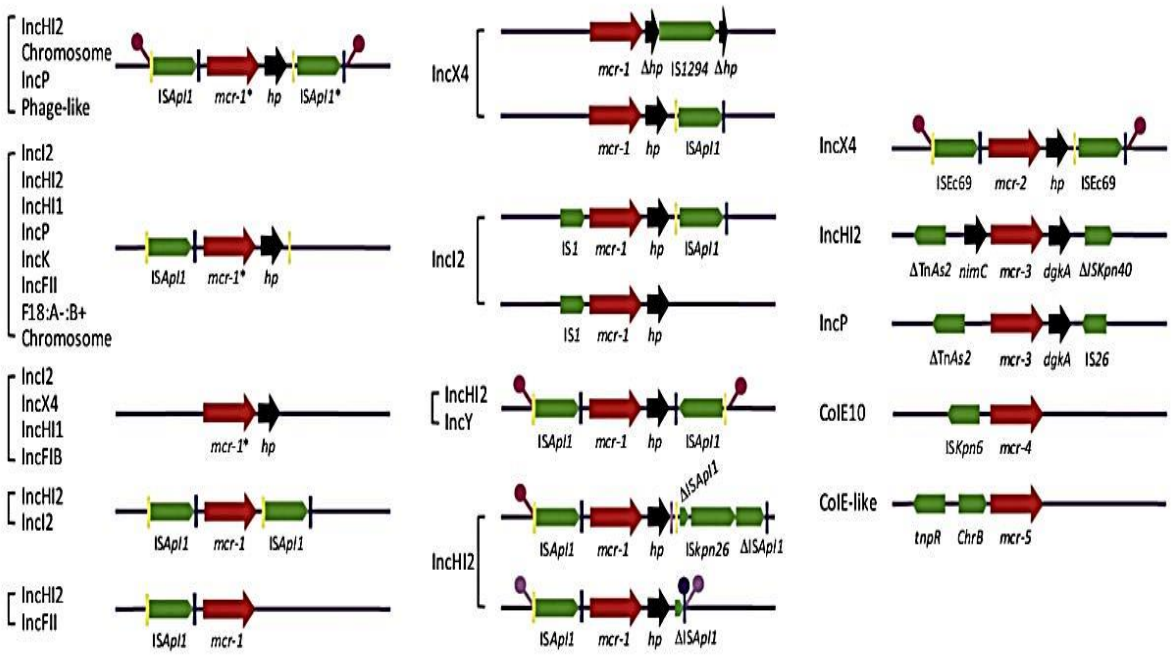


Figure 4: Diversity amongst genetic contents of five *mcr* -like gene (*mcr-1,mcr-2,mcr-3,mcr -4,mcr-5*) (Sun and al, 2018).

VI. Prevalence of colistin resistence in the environment (animals, water, soil)

Reports have shown that antibiotic resistance genes and the same genetic platforms (plasmids, integrons) which are currently present in human pathogens, have been detected in pristine environments and in humans and animals populations that have never been in contact with antibiotics. This suggests that antibiotic resistance genes which have integrated in successful gene-transmission elements could persist and spread in the environment even in the absence of antibiotics (Martinez and al., 2009).

Since colistin determinant emerging from any part of the globe can rapidly spread worldwide by international travel (even short distance travel) and food trade, there is a need for increased surveillance of *mcr* genes in environmental reservoirs, especially in Africa where the use of COL is largely uncontrolled, and sanitation is poor, and South America where public and environmental sanitation is also considered suboptimal. Indeed, it is evident that by horizontal/lateral and vertical transfer, *mcr* genes (*mcr-1, mcr-2, mcr-3, mcr-5, mcr-7,* and *mcr-8*) have spread widely into diverse environmental niches (Figure 5).

Thus, these ecosystems constitute underestimated vast reservoirs (‘phantom resistome’) of these *mcr* genes (Anyanwu and al , 2020).

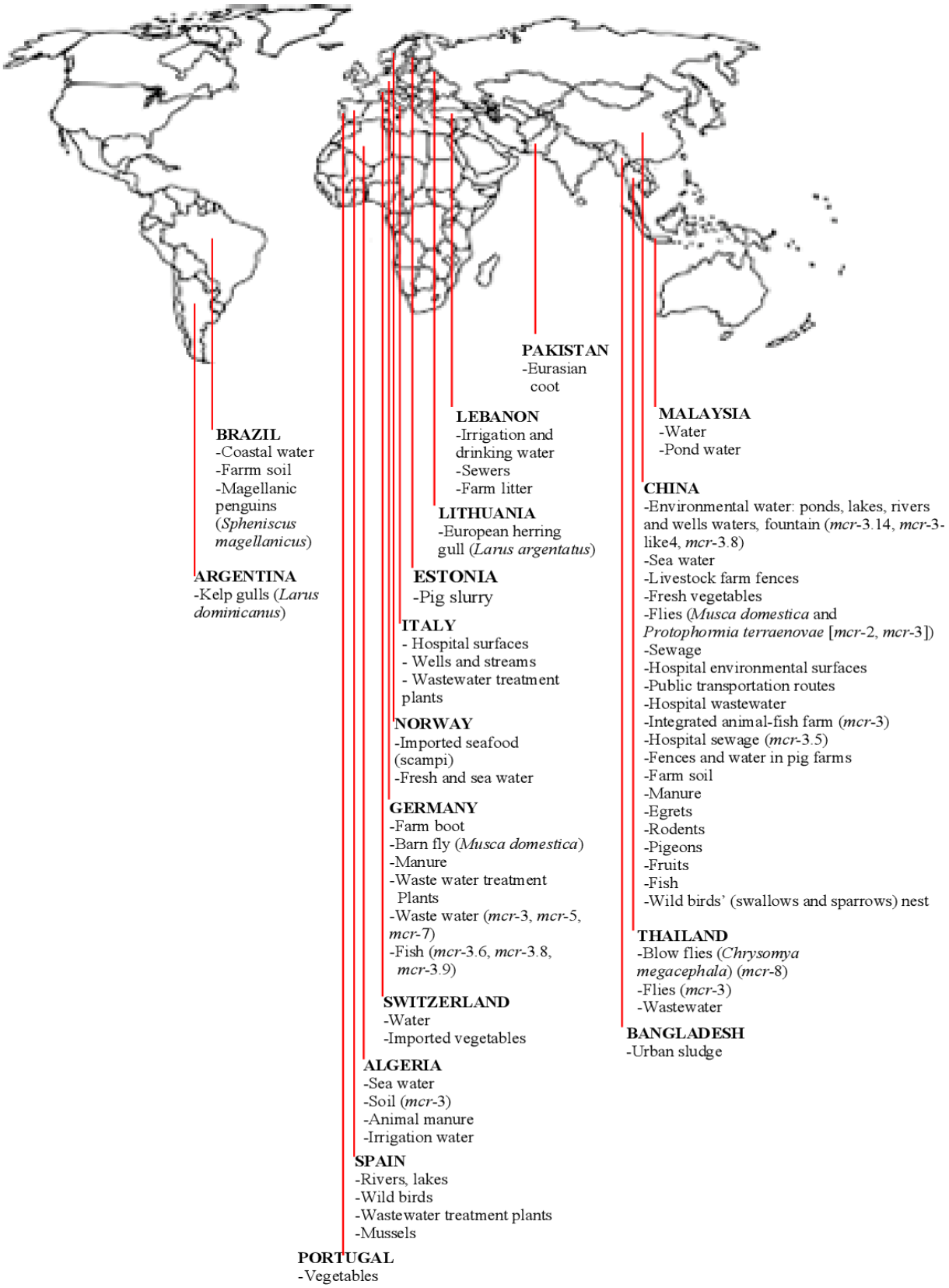


Figure 5: Geographical of distribution of the *mcr* gene detected in environmental reservoirs (Anyanwu and al, 2020).

VI.1. Animals

Colistin has been used extensively for decades in animal husbandry, not only for the prevention and therapy of infectious diseases but also for growth promotion animals, in particular sheep and pigs, would be a source of dissemination important of the *mcr* genes is widespread. These assumptions are based on the fact that these genes have been identified in several isolates from animals and food products: pork, poultry, turkey, calf (kemps and al, 2016).

This antibiotic is widely used in veterinary medicine especially in China and Southeast Asia for the treatment of infections caused by GNB in pig and poultry populations (Burowand and al, 2019). The intensive use of colistin in veterinary medicine, for decades has resulted in selection pressure in *Enterobacteriaceae* (Andrade and la., 2020) and may explain the high prevalence of these resistances in animals, In November 2015, the first discovery of a mobile colistin resistance gene (*mcr1*) was reported in food animals and humans from China (Liu and la., 2015). Shortly thereafter, *mcr1* was identified in more than 40 countries on five continents (Figure 5).

European countries and China are recognized by the frequent use of Antibiotics in chicken and pig farming, the treatment of infections and symptoms caused by enterobacteria which colonize farm animals with colistin is a potential route of the introduction of the *mcr* gene which confers resistance to colistin, The presence of mobile colistin resistant gene has been identified among *E. coli* strains Isolated from the livres of chickens displaying symptoms of colibacillosis in Tai'an (song and al .,2019). Also breeders through their contact with animals or farm materials can they spread these genes to other animals such as companion animals (Simmen and al., 2016).

In France, in production animals, the carrying rate of *E. coli* carriers of *mcr-1* gene is variable: 0.5% in pigs in 2013, 2% and 6% in chickens and turkeys respectively in 2014 (.Guyomard and al., 2016)

Migratory birds that migrate for long distances in shorter duration may eliminate this gene instead, Where in Brazil has been detected *E. coli* carrying Incx4 plasmid mediated *mcr1* and $bla_{CTX\text{-}M\text{-}1}$in infected migratory magellanic Penguins (Edgarthe , 2019).

In addition, the literature's data clearly show a dissemination of the *mcr1* gene in the animal world and more particularly in cattle (sheep, cows) (Liu and al., 2016), Researchers

studied 400 cows suffering from mastitis on 23 Greek farms, 89 samples were positive for colistin from half of the farms and about 6 samples showed multidrug resistance and contained the *mcr1* gene (Nagy and al., 2012).

Animals live in aquatic environments such as fish and Moluccas are reservoirs of the *mcr* genes, because aquatic environments determining as a source of ARG for example studies reported that in aquatic Strain of *Shewanella algae* from *Haliotis diversicolor*, a marine gastropod mollusc was resistant to colistin by harbouring the pmrCAB operon (Huang and al .,2018)

African fauna recognized as a reservoir of the AMR resistance gene, this is not surprising because while the use of COL in humans is rare in Africa, its use in animal husbandry is largely unregulated (Maamar and al., 2018). Thus, there is a strong lihood that in Africa MCR was born in the veterinary sectors from where they diffused towards other ecosystems, like other drugs in most of the African countries trials except for the South Africa COL is an over-the-counter medicine sold and dispensed by non-professionals without the supervision of a veterinarian, the majority of Africans including livestock farms, have poor knowledge of ATB (Van and al., 2020).

Unfortunately unsanitary animal slaughter techniques are employed in the majority of house slaughter in Africa, and even worse among Africans especially those in rural areas humans live in very close contact with their livestock (Alonso and al., 2017). For example, in Tunisia, the *mcr1* gene has been detected in food animals (chickens, camels and cattle) and food products (chicken meat, beef and cow's milk) (Grami et al., 2016; Hassen et al., 2019; Saidani et al., 2019, Hassen et al., 2020)

On the other hand, wild animals (funec) captured in Sudan were vectors for the export of *mcr1* to China (Feng et al., 2019). As well as in Egypt the *mcr2* gene has been detected in migratory birds and resident birds, in addition to *mcr9* in beef, chicken and cheese (Elnahriry and al., 2016,Sadek and al., 2020)

Likewise, in Algeria, a study showed the presence of the gene *mcr-1* in a strain of *E. coli* isolated from chicken in 2015 (Olaitan and al., 2016). Also in the same country, in the forests of Toudja (Bejaia, Algeria) a primate, *Macaccas sylvanus* was colonized by *E.coli* carrying the *mcr1* gene after the collection and analysis of stool samples (Bachiri and al, 2018).

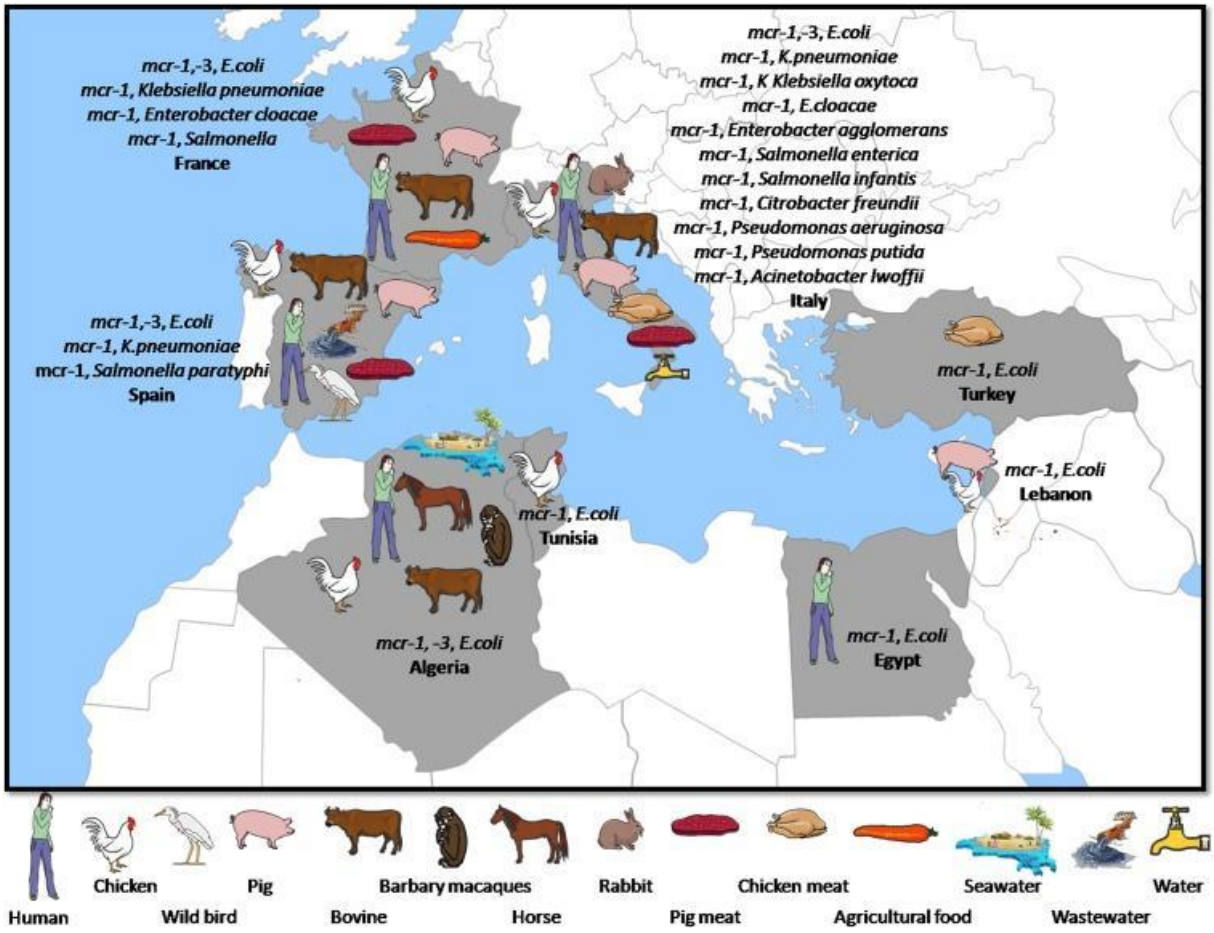


Figure 6 : Distribution of mcr-1 and mcr-3 genes in the mediterranean basin (Touati and al., 2020)

VI.2. Water

The emergence and dissemination of colistin resistance is an urgent threat to public health worldwide (Olaitan and al., 2014). Resistance to colistin has been observed in hospital and non-hospital settings, particularly in the aquatic environment (Olaitan and al., 2014; Baron and al., 2016) such as wastewater which has been a source of emerging pathogens and antibiotic resistance (Lood and al., 2017)

Wastewater treatment plants and water from canals were reported as reservoirs of *mcr1* in particular in European countries (Hembach and al., 2017), as expected the abundance of improved protocols used in the treatment of wastewater causes problems on public health,especially at the level of hospitals and health services. Because hospital environments containing many ATBs can stimulate resistance to colistin, for example several Chinese studies have noted that hospital wastewater can contain clinical samples

such as feces, blood fluids, decomposed tissue is a problem. source and potential route of spread of COL resistance (Wang and al., 2018).

Aquaculture water discharged or used for irrigation can introduce COL resistance or *mcr* gene and unmetabolized antimicrobials into aquatic systems (surface water, groundwater) (Baquero and al., 2008). Which was reported in Euroupe, in 2016, a multiresistance *E. coli* strain carrying *mcr1* was detected among 74 ESBL Enterobacteria isolated from rivers and lakes in Switzerland (zurfuh and al., 2016)

Likewise, sea water and public beaches polluted by anthropogenic and agricultural waste, suggesting that it is a reservoir for multiple resistance genes (Jorgensen and al., 2017; Fernandes and al., 2017). Wildlife in Africa has also been associated with disseminating *mcr* genes into the environment (surface waters) and the human population (Ahmed and al., 2019).

Improper disposal of livestock/slaughterhouse wastes into the environment and their use as organic fertilizer in farmlands and aquaculture can spread *mcr* genes into the environment. For example, *mcr1* has been detected in irrigation and sea waters in Africa (Drali and al., 2018, Touati and al., 2020). Too wastewaters are established sources of new emerging pathogenie antimicrobial resistant organism. Thirty one MDR *E.coli* carrying *mcr1* were isolated from municipal wastewater effluent in South Africa (Anyanwu and al., 2021). Another african study reported that GNB whose bacteria carrying *mcr1* and *mcr5*. Resistance to colistin were isolated from all water source such as the plankenburg, Eerste and Berg rivers and in strom water from muizenberg (Snyman and al., 2021).

At Mediterranean sea levels, studies have shown that two *E. coli* resistant to the tigecycline of the ST115 and ST23 strains carrying *mcr1.1* on InCHI2A and *mcr 1.5* on the plasmid IncI2 respectively were detected among the isolates resistant to colistin in polluted seawater (Olaitan and al., 2014; Chen and al., 2017). Another study has shown that a strain of *E. coli* carrying the *mcr1* gene has been detected in sea water From 62 beaches of Algeries coast (Drali and al., 2018). Agricultural and industrial waste can cause pollution due to improper waste disposal in water bodies of the Mediterranean Sea

VI.3. Soil

By definition, Soil serves as the primary nutrient base and habitat for plants and organisms, and plays a vital role as a giant bioreactor for degrading pollutants and facilitating nutrient transformation (Martinez and al., 2009), Despite the widespread and growing evolution of AMR, limited focus has been placed on the role of soil in propagating resistance (Ashbolt and al., 2013).

The use of antimicrobials as biocides on plants potentially results in colonization of plants and soilby antimicrobial resistant organisms (Maillard, 2018). Too Use of animal manure and insufficiently-treated/untreated sewage sludge as fertilizer in farmlands and aquaculture are a potential routes of introduction of colistin resistance into the soil (Thanner and al.,2016; Maillard.,2018)

Countries in the United States have reported the presence of the *mcr* gene in soil, resistance to COL in soil does not necessarily occur only when livestock manure has been used.In a Brazilian study, *mcr1* was detected in soils from vegetable production areas that received non-composted poultry litter as organic fertilizer as well as in native vegetation areas without livestock manure (Oliveira and al., 2019).

Although not surprising, the *mcr1* was more abundant in the fertilized vegetable production area than in the native vegetation area, suggesting that non-composted poultry litter is a potential source of *mcr1* in the soil(Oliveira and al ., 2019)

Another study reported that *mcr1* positive ESBL producing *E.coli* Strain (S802) was detected from kall corps, this strain belonged to serotyp O25: H4 and was phylogenitically related to human avian and swine (Lopes and al., 2021)

From china the presence of colistin in 12 of 53 (22,6%) ESBL producing Enterobacteriaceae isolates of 96 soil sample from farming soil in Shandong province (zheng and al., 2017).The soil constitue a source for dissemination of these genes to botanical, plants (fruits, vegetables, and grasses) could get contaminated with colistin-resistant organisms emanating from soil (Heaton and al.,2008; Hö and al.,2018)

Between May 2016 and March 2018 in Algeria, forty environmental samples were collected at eight agricultural sites in the Oran region from the 40 samples, colistin resistant isolates were isolated from agricultural soil, irrigation water and manure, eight

isolates were identified as *E.coli* including six and two isolates ,carrying the *mcr1* and *mcr3* gene respectively (Touati and al., 2020).

PART II

Methods to detect colistín resistance

The emergence of antibiotic resistance of clinical interest usually conduces to the development of new tools in clinical microbiology laboratories. Currently, the detection of carbapenemase-producing bacteria is well determined, combining specific culture media, phenotyping testing, antibiotic susceptibility testing, and molecular biology. As colistin resistance is a recent global phenomenon, the implementation of rapid and reliable screening tools to detect and analyze colistin resistant pathogens in such a way as to isolate the patient and adapt the treatment is a necessary approach. Moreover, hetero resistance to colistin is a common phenomenon that is widely underestimated, requiring specific methods. Here, we propose an overview of all the screening and analysis methods developed to assess colistin resistance among bacterial pathogens causing infectious diseases.

I. Phenotypic detection methods

I.1. Broth Microdilution (BMD)

The objective of Broth Microdilution is to determine the minimum inhibitory concentration which corresponds to the smallest concentration of colistin that inhibits bacterial growth visible after 16-20 hours incubation at 35°C (Jayol, 2018).

In 2016, both EUCAST and the Clinical and Laboratory Standards Institute (CLSI) recommended the International Standard Organization (ISO) 20776 standard broth dilution method for testing of the MIC values of colistin (Stefaniuk and Tyski,2019).

BMD panels were prepared extemporaneously in 96-well sterile polystyrene microplates (figure7). Dilutions of colistin ranging from 0.125 to 128 mg/L were made in cation-adjusted Mueller Hinton broth, without addition of polysorbate 80 (Tween 80) and with a final concentration of 5 and 105 CFU/mL of bacteria in each well. This procedure was performed in triplicate in separate experiments, and the minimum inhibitory concentrations were read after 16 to 20 hours of incubation at 35± 2%C in ambient air. Results were interpreted according to the EUCAST breakpoints (Jayol and al, 2017).

However, the method of Broth Microdilution is tedious, requires preparation manual antibiotic stock solutions can lead to errors and therefore cannot be used by most routine microbiology laboratories. Despite these drawbacks, the method of dilutions in liquid medium remains the reference method to determine MICs due to its reproducibility, reliability, and the possibility of automation (Jayol, 2018). Moreover, this method exhibits limitations for assessing heteroresistance. Indeed, the presence of resistant subpopulations can give uninterpretable results due to the presence of skipped wells and has been described for the *Enterobacter* species (Bradet and Rolain, 2018).

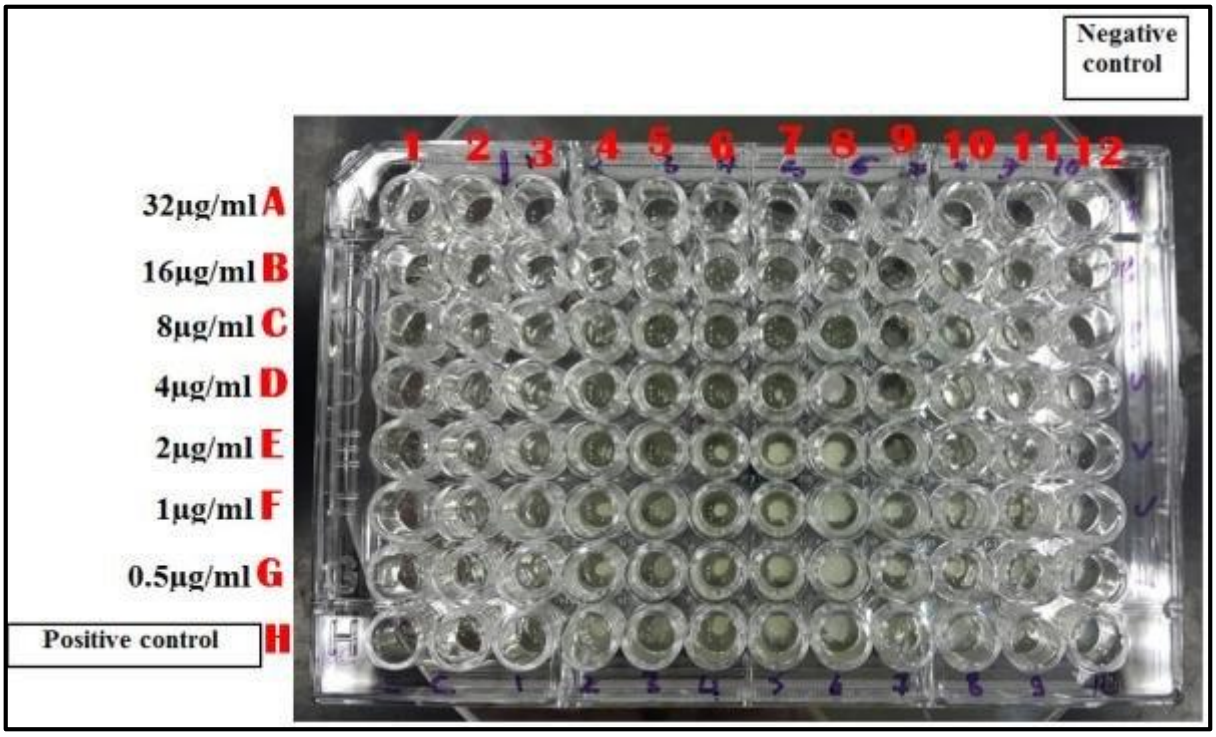


Figure 7 : Broth microdilution plate

I.2. Super polymyxin

The SuperPolymyxin medium (Elitech Microbio, Signes, France) was developed and intended to specifically detect colistin-resistant strains, including those with a low MIC of colistin and harboring the *mcr-1* gene (Bradet and Rolain, 2018). It is a selective medium for polymyxin-resistant Gram-negative bacteria that is based on eosin methylene blue (EMB) agar. The medium was developed with the optimal colistin concentration of 3.5 µg/ml, 10 µg/ml of daptomycin (to inhibit potential growth of Gram positive strains) and 5 µg/ml of amphotericin B as an antifugal. The use of eosin Y and methylene blue dyes helped distinguish lactose-fermenters (dark brown to purple) from non-fermenters

(colourless). This medium distinguishes lactose fermenting *E. coli* (metallic green sheen) from other *Enterobacteriaceae*, including non fermenting *E. coli* (dark brown to purple) (Leshaba and al,2020).

Table II: Methodology of super polymyxine (Flyer,2019)

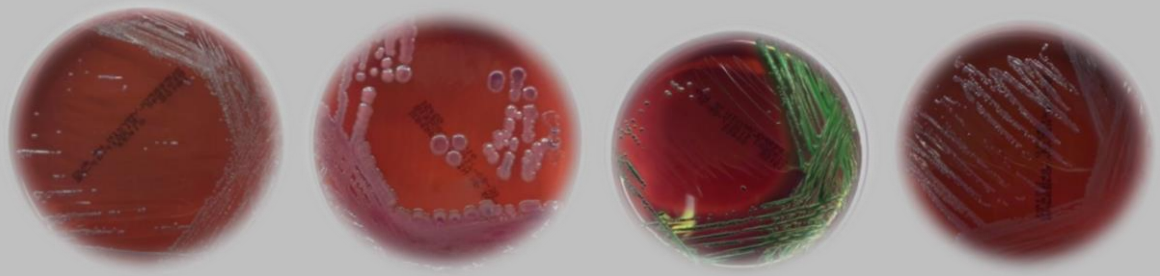
Samples
Bacterial colonies + rectalswabs
Procedure
• **Direct streaking from rectal swab transport medium,** • **preparation of a 0.5 McFarland suspension brothneeded for colonies** • **Incubation 24-48h at 37°C** • **Aerobic conditions**
Results
lactose fermenters : darkblue-brown colonies **Non fermenters : colorless or light lavender.** ***Escherichia coli* : colonies displaying a characteristic metallic green sheen**      ***A. baumannii K. pneumonia*** ***E. coli P. aeruginosa***

Figure 8: Identification of colistin resistant BGNs using the medium superpolymyxin.

I.3. CHROMagar™ COL-APSE

The second colistin-resistance agar-based screening medium to be developed after the SuperPolymyxin was the CHROM agar COL-APSE media, that also detects colistin-resistance in *Enterobacteriaceae* and Gram-negative non fermenting bacteria (Sekyere, 2018)

CHROMagar™ COL-APSE by CHROMagar (Paris, France) is the first selective medium designed to detect and differentiate all *Acinetobacter spp.*,*Enterobacteriaceae, Pseudomonas spp*. and *Stenotrophomonas spp.* (Leshabaa and al,2020).A major advantage of CHROMagar COL-APSE over SuperPolymyxinis the former'sability to identify Gram-negative non-fermenters more efficiently (figure 9) (Abdul Momin et al., 2017). The medium's composition (Table 1, annexe 1), is such as to prevents warming of *Proteus spp.* and improve upon the differentiation and identification of more species, dark-pink to reddish (*E. coli*), metallic blue (*Klebsiella*, *Enterobacter* and *Serratia spp.*) etc. While *E. coli* is the only identifiable species on SuperPolymyxin (Sekyere, 2018)

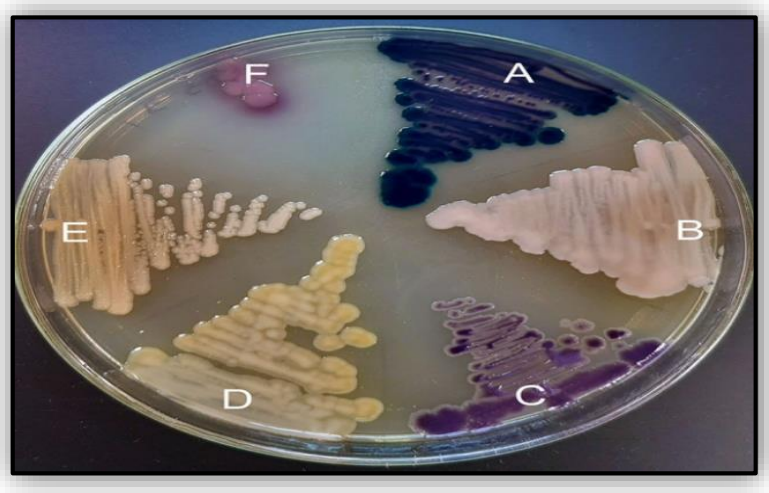


Figure 9: Colistin-resistant organisms grown on CHROMagar COL-APSE agar for 48 h; (A) *Aeromonas hydrophila* ; (B) *Alcaligenes faecalis ;* (C) *Hafnia paralvei* ; (D) *Myroides odoratus*; (E) *Pseudochrobactrum spp.* ; (F) *E. coli* (Bean, 2020)

I.4. LBJMR medium

Lucie-Bardet-Jean-Marc-Rolain (LBJMR) medium, was also developed to detect all the colistin-resistant bacteria, including those harboring *mcr1* genes. The LBJMR medium

presents the advantage of being versatile, combining colistin-resistant and vancomycin-resistant bacteria (Bardet and al, 2017) (Table III).

The LBJMR medium is a multipurpose selective medium which makes it possible to identify bacteria of interest from clinical samples and to isolate contaminated patients in hospital settings. This is a simple medium that could be easily used for screening in clinical microbiology laboratories (figure 10)

This medium was developed by adding colistin sulfate salt at a low concentration (4 µg/mL) and vancomycin (50 µg/mL), with glucose (7.5 g/L) as a fermentative substrate, to a Purple Agar Base (31 g/L)(Leshabaa and al, 2020)

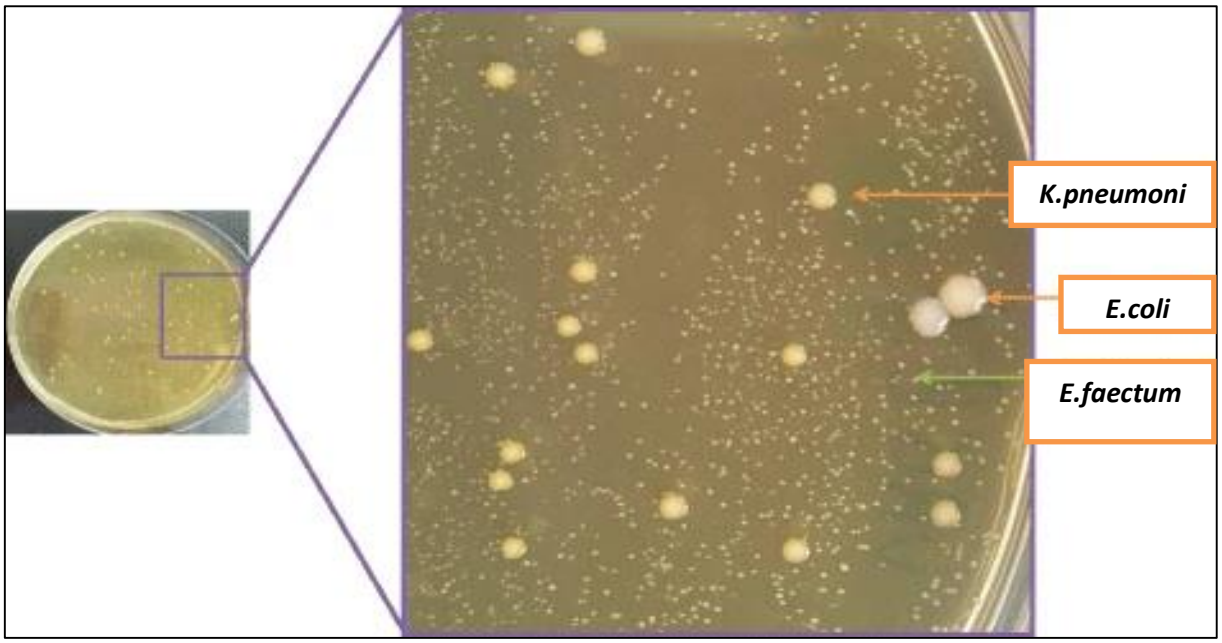


Figure 10: Aspect of the differents types of colonies on LBJMR after culture of a clinical sample (Bardet and al, 2017).

Table III : Performance of LBJMR medium (Bardet and al,2017)

Criteria	LBJMR medium
Isolates screened	Colistin-resistant Gram-negatives: - Enterobacteriaceae, including those harboring the *mcr-1* gene - Non-fermentative Gram-negative colistin-resistant strains, including those involves in cystic fibrosis samples - Vancomycin-resistant Gram positives, including *Enterococci*
Aspect of colonies	Yellow on purple agar: 2–3 mm for *Enterobacteriaceae*, 0.1–1 mm for *Enterococci*
Incubation Culture of samples Isolates analysis	Aerobic atmosphere, 37 °C, 24H (sterile at 48H) Direct on LBJMR, no previous decontamination Colonies can be picked directly from primary cultures on LBJMR for analysis: - MALDI-TOF identification - Antibiotic Susceptibility testing - PCR screening for resistance genes.
Avoid contamination	- Inhibition of *Proteus* swarming - Inhibition of yeast possible by adding amphotericin B

I.5. Rapid NP polymyxin

The rapide pollymyxin NP test is easy to parform, rapid, sensitive and specific .It detects polymyxin rasistant and susceptible isolates from any enterobacterial species (http://doc.rero.ch). It is based on a simple pH test, and detection of colistin resistance is obtained by a color change within 2 hours (Bradet and Rolain, 2018).

This test is based on the detection of the glucose metabolization related to bacterial growth in presence of a defined concentration of colistin. Formation of acid metabolites consecutive to the glucose metabolization was evidenced by a color change (orange to yellow) of a pH indicator (red phenol)(http://doc.rero.ch)(figure 11).

Rapid NP polymyxin test is commercially available (ELITech Group, Puteaux, France) and can also be performed in-house with the preparation of two solution (Humphries and al. 2014 ;Bialvaei and al. 2015). The in-house rapid polymyxin NP test is prepared with stock solutions of polymyxins and a rapid polymyxin NP solution, which consists of cation-adjusted Mueller Hinton broth powder, phenol red indicator and D(+)-glucose (Nordmann and al ,2016) (annexe 2).

Compared to the broth microdilution (BMD) susceptibility testing method, agreements were excellent to detect *mcr-1* and *mcr-2* strains. The rapid polymyxin test has a good sensitivity to detect *Hafnia* sp. colistin-resistant isolates but failed to detect *Enterobacter* sp. isolates, surely due to their heteroresistance to colistin. This test has to be evaluated with nonfermentative colistin-resistant strains, such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (Bradet and Rolain, 2018).

I.6. Micromax Assay

Micromax Assay for *A. baumannii*. The Micromax assay is based on the detection of DNA fragmentation and cell wall damage in the presence of colistin. Bacteria are incubated for 60 min with 0.5 µg/ml of colistin (Jayol and al, 2016), trapped in a microgel, and then incubated with a lysis solution to remove weakened cell walls. The presence of DNA fragments is detected after staining by SYBR Gold fluorochrome and observed by fluorescence microscopy. Resistance corresponds to ≤11% of bacteria with cell wall damage. This method is rapid (3h30 min) and showed an excellent sensitivity for the

detection of colistin resistance but it is not specific for determining the resistance type (Tamayo, 2013).

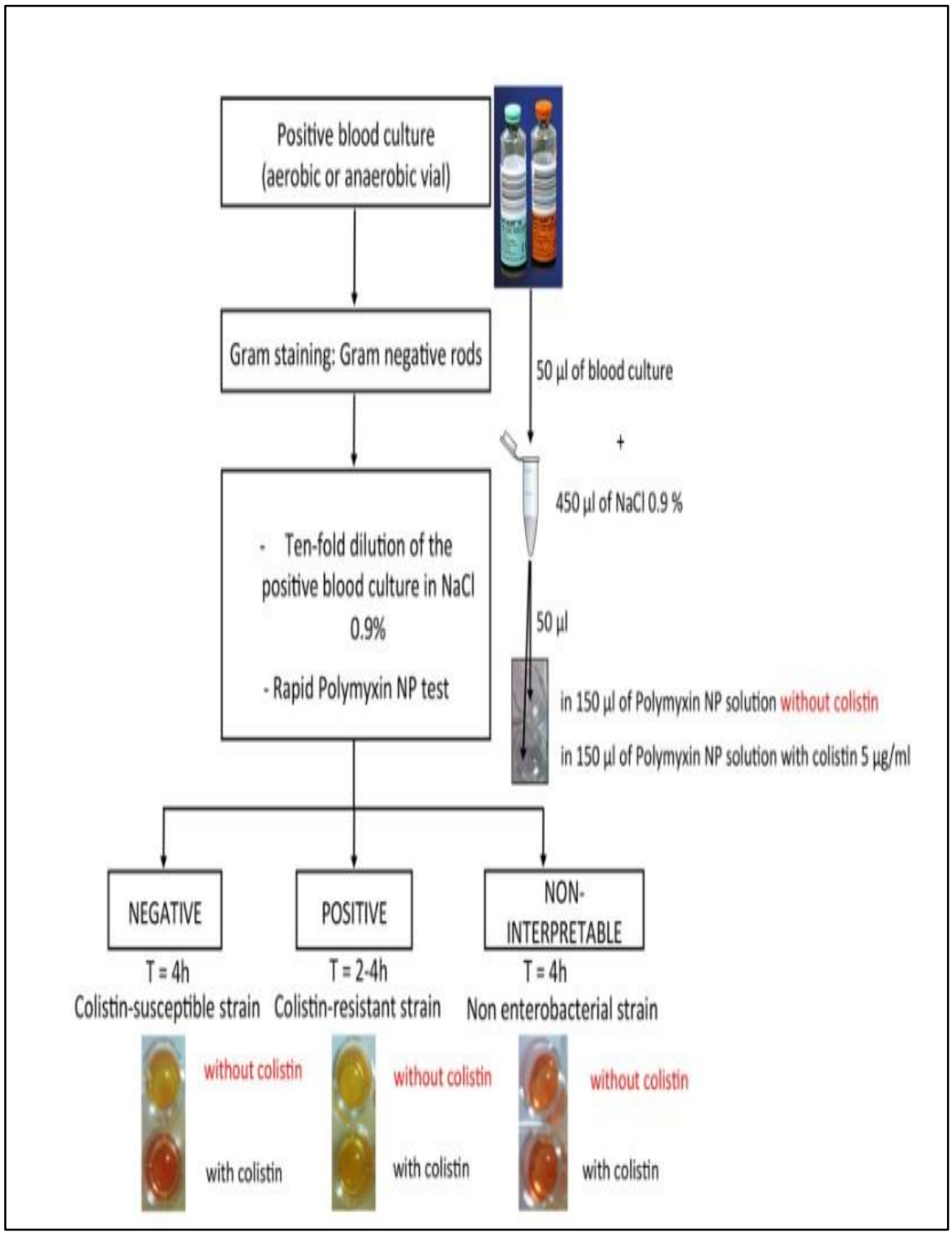


Figure 11 : Strategy for identification of colistin-resistant *Enterobacteriaceae* from blood cultures using the rapid polymyxin NP test. T, time (Jayol and al, 2016)

II. Genotypic methods

As it understands of colistin resistance mechanisms improves, so will the concordance between phenotypic and genotypic test results. As for many other classes of antimicrobial agent, molecular testing may eventually offer an alternative to phenotypic testing for the surveillance of colistin resistance. Nevertheless, if the results are intended to guide clinical management, inference of phenotype based solely on a genotypic result may be valid only when the genotypic result is positive (i.e. mechanisms or genes detected), with a cautionary note that the resistance phenotype is likely but not guaranteed. If the results of the genotypic test are negative, no inference should be drawn about phenotype (WHO, 2018).

II.1. Recombinase polymerase amplification (RPA)

A novel isothermal amplification approach that employs recombinase polymerase amplification (RPA) has been demonstrated. This method has a number of advantages. RPA can be performed in less than 30 min at body temperature. RPA technology not only does not depend on precision thermal cycling instruments, but also has the advantages of fast response, simple operation, high specificity and high sensitivity. This technology has been reported in food safety, biological detection, in vitro diagnostics and other fields (Xu and al., 2018).

In study conducted by researchers they used basic RPA (B-RPA) and RPA with lateral flow (LF-RPA) on 23 genomic DNA extracted from 20 *mcr1* positive and three *mcr1*-negative *Enterobacteriaceae*. The primers for the B-RPA assay were designed by Clone Manager 8 and validated on three *mcr1* positive and one *mcr1* negative DNA samples. The B-RPA was based on the Twist Amp Basic kit reaction system, which was incubated at room temperature for 30 min, after which the amplicons were extracted by phenol/chloroform solution or purified using an amplicon purification kit. The LF-RPA reaction required primers and a probe, which were labelled with biotin and fluorescence. The LF-RPA was based on Twist AmpNfo kit reaction system, which was incubated as described for the B-RPA. The amplification products for the LF-RPA were diluted at 1:50 with running buffer, after which a down stream operation was carried out. The results for the B-RPA assay were read by agarose gel electrophoresis, whereas the results for the LF-RPA assay were visually read using Hybridetect 2T dipsticks. A positive *mcr1* detection by

LF-RPA was demonstrated by two purple bands at the test line and the quality control line (Leshabaa and al,2020) .

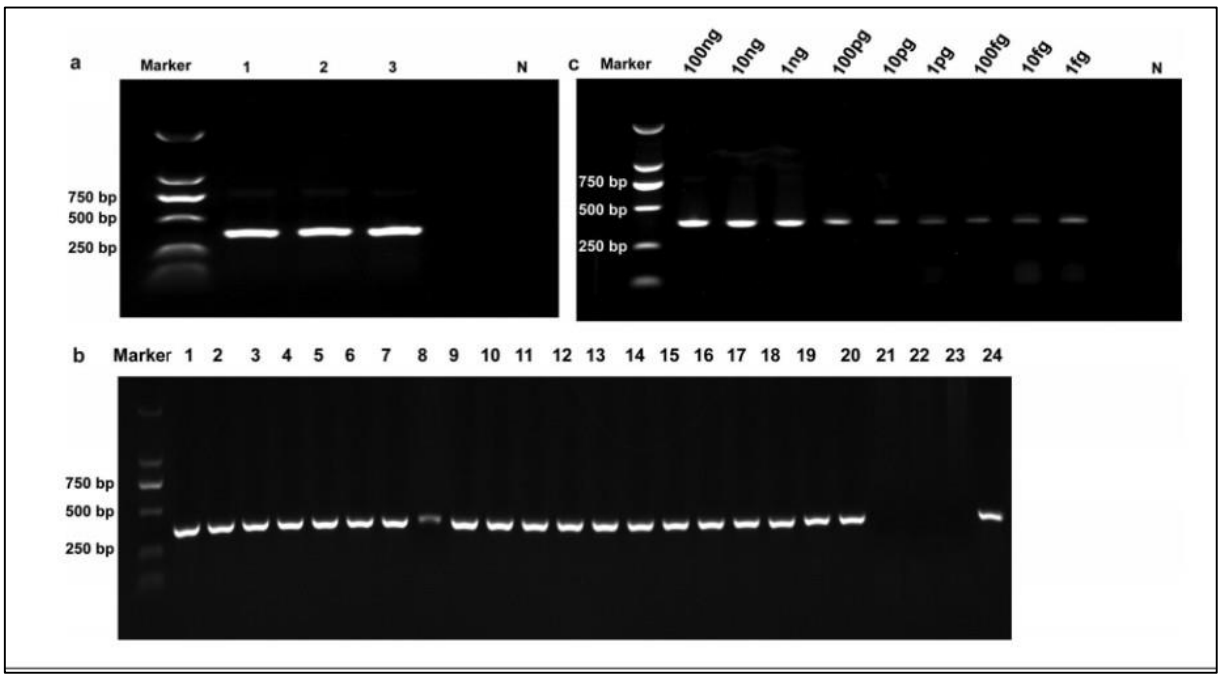


Figure 12 : Results for the B-RPA (recombinase polymerase amplification with agarose gel electrophoresis assay (Xu and al, 2018).

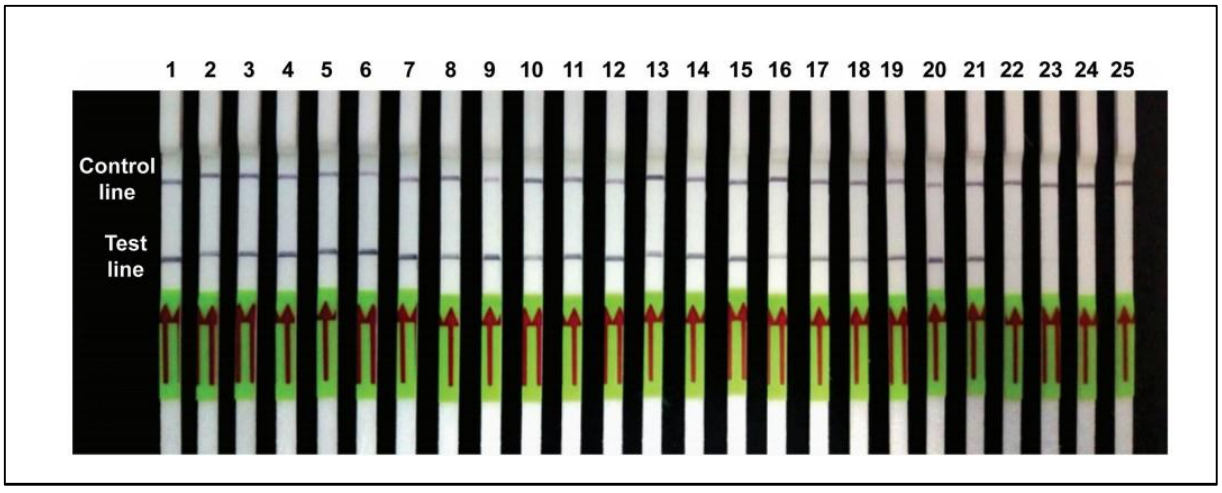


Figure 13 : Results for the LF-RPA assay positive strains (Xu and al, 2018).

II.2. Loop mediated isothermal amplification (LAMP)

Loop mediated isothermal amplification (LAMP) is a nucleic acid amplification method that allows autocycling strand displacement DNA synthesis at constant temperature using Bst DNA polymerase (Zhong, 2019). The use of LAMP for detecting *mcr1* gene was first described by two studies (Imirzalioglu and al, 2017; Zou and al, 2017). Zou et al (2017) established a LAMP assay for the detection of *mcr1* gene from cultured bacteria and spiked human stools (Zou and al, 2017).

The LAMP reaction was carried out in a 25 µL reaction mixture that contained 12.5 µL LAMP-Reaction Mix [20 mM Tris-HCl (pH 8.8), 10 mM (NH4)2SO4, 8 mM MgSO4, 10 mM KCl, 0.8 M betaine, 0.1% Tween-20, 1.4 mM deoxy-ribonucleotide triphosphates (dNTP)], 1 µL Bst 2.0 polymerase (New England Biolabs, 8,000 units/mL), 1.25 µL primer mix (2 µM each of FIP and BIP, 0.25 µM each of F3 and B3, and 1 µM each of LF and LB), 8.25 µL nuclease-free water, and 2 µL DNA lysate. The mixture was incubated for 60 mins at 64°C in a heated, thermostatically controlled water bath (Zhong et al ,2019).

A PCR assay was performed to compare its sensitivity and the clinical detection rates with those of the LAMP assay, the cycling conditions were as follows: 3 mins at 95°C; 30 cycles of 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C; 5 mins at 72°C. The PCR products were analyzed electrophoretically on a 2% agarose gel, followed by ethidium bromide staining. Images were obtained using the (BioRad, Hercules, CA, USA) (Zhong et al, 2019).

II.3. PCR Amplification and Sequencing to Detect Gene Mutations

Molecular biology methods are the most sensitive for determining antibiotic resistance by assessing the presence of resistance genes or mutations conferring resistance. These methods are complementary to the phenotypic techniques and confirm the resistant status of bacterial isolates. The main mutations for *Enterobacteriaceae* species are located on genes coding the two-component systems PmrA/PmrB and PhoP/PhoQ (Figure14). Specifically, mutations in the mgrB gene the negative feedback regulator of PhoPQ notably with the presence of insertional sequences, appeared to be the main resistance mechanism observed in *K. pneumoniae* strains. These colistin resistances are not based on

drug-modifying enzymes or the acquisition of a resistance gene which could be easily detected. Screening of potential mutations on these chromosomal genes is done by amplification and sequencing, takes 3 days, and requires that all genes are tested. Sequenced amplicons are then compared by the BLAST tool against the NCBI database to screen possible mutations compared to wild-type genes (Bardet and Rolain, 2017).

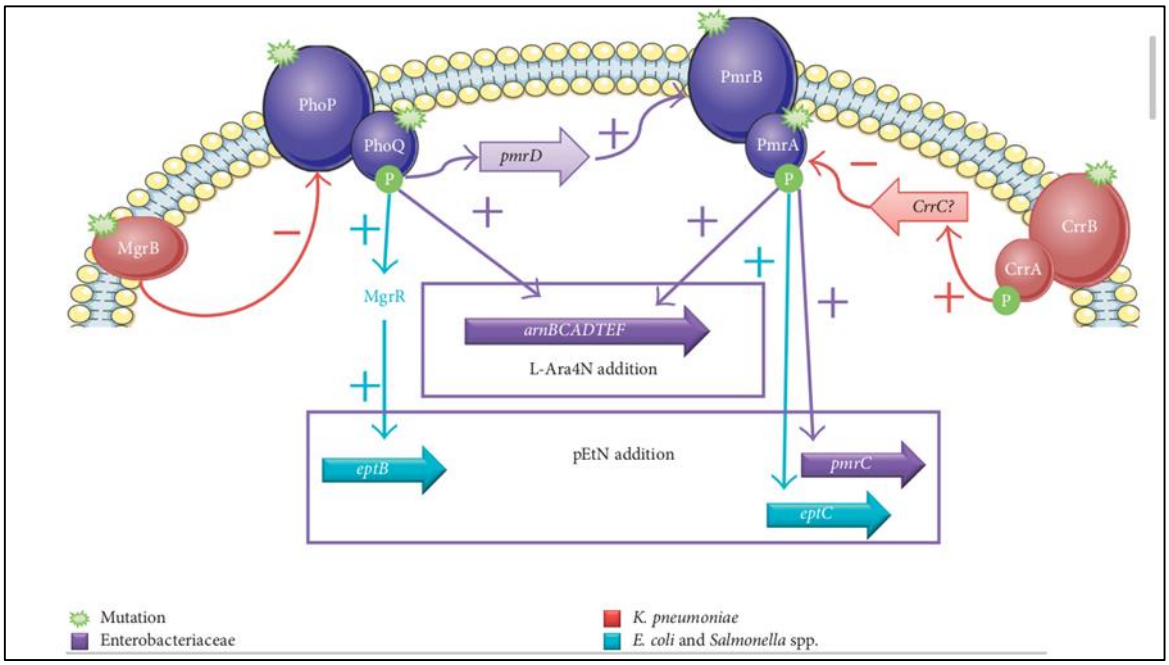


Figure 14: Molecular mechanisms of acquired resistance to polymyxins. L-Ara4N: 4-amino-4-arabinose; pEtN: phosphoethanolamine (Bardet and Rolain, 2017).

II.4. Microarray

Microarray technology is a developing technology used to study the expression of many genes at once. It involves placing thousands of gene sequences in known locations on a glass slide called a gene chip. A sample containing DNA or RNA is placed in contact with the gene chip. Complementary base pairing between the sample and the gene sequences on the chip produces light that is measured. Areas on the chip producing light identify genes that are expressed in the sample (https://www.genome.gov/genetics-glossary/Microarray-Technology).

Microarrays are a technology that basically miniaturizes processes that have been used in molecular genetics laboratories for years. They allow detection of DNA or RNA

molecules by hybridizing or sticking to target DNA molecules or RNA molecules on a glass slide, with detection of that adherent DNA or RNA molecule by various labels or dyes that allow them to be seen under a microscope.(https://www.genome.gov/genetics-glossary/Microarray-Technology)

The commercial CT103XL microarray was confirmed to simultaneously detect *mcr1/2* and β-lactamase genes with accuracy, although it failed to detect *mcr3*, which shares 45% and 47% identity to *mcr1* and *mcr-2*, respectively (Bernasconi and al., 2017).

II.5. Real-time PCR

This method represents a set of sensitive, rapid and effective assays for the screening of colistin resistance directly from the environment (Tolosi and al, 2020).

Nijhuis et al (2016) were the first to design a real-time PCR assay for detecting *mcr1* from clinical isolates using self designed primers and probes. The assay was validated on 26 *mcr1* positive *E. coli* isolates, where the presence of *mcr1* was detected in all 26 isolates. Additionally, the assay was evaluated on spiked stool samples and the efficiency of the PCR was 102.6% and the LOD was 3-30 cfu/reaction (Nijhuis et al 2016). However, *mcr1* genes were not detected in other colistin-resistant strains i.e. *Klebsiella, Enterobacter, Pseudomonas, Acinetobacter* etc. (Leshaba and al,2020).

Another study in which Stools (n=88) from 36 volunteers were analysed. To isolate *mcr1*-producing *Enterobacteriaceae*, samples were enriched overnight in Luria-Bertani (LB) broth containing 2mg/L colistin and were then plated on selective agar plates with 4mg/L colistin. A SYBR(®) Green-based rt-PCR targeting *mcr1* was then designed. For method validation and to establish the limit of detection (LOD), total DNA was extracted from *mcr1* -negative and *mcr1* positive *E. coli*. rt-PCR was also performed with DNA extracted from 88 native stools and after enriching them in LB broth containing colistin. The results showed that three unique volunteers resulted colonised with *mcr1* -harboring *E. coli* strains. For culture isolates, rt-PCR exhibited a LOD of 10 genomic copies/reaction, with both sensitivity and specificity of 100%. Nevertheless, when testing native stools, only two of the three *mcr1* -positive specimens were detected. However, after enrichment in LB broth containing colistin, rt-PCR was strongly positive for all culture-positive samples. The average cycle threshold was 22, granting rapid and confident detection of

positive specimens within 30 cycles. No false positives were observed for the remaining 85 culture-negative specimens (Dona and al,2017).

A recent study described a multiplex SYBR Green real-time PCR assay for the simultaneous detection of *mcr1*, *mcr2,* and *mcr3* genes. also, a multiplex PCR assay for detection of the five *mcr* genes: *mcr-1, mcr-2, mcr-3, mcr-4*, and *mcr-5,* was developed in order to obtain sequential amplicons with a size difference of 200 bp, allowing their fast and simultaneous detection on agarose gels (Bradet and Rolain, 2018).

Conclusion

Colistin is one of the few antibiotics still active against Enterobacteriaceae producing carbapenemase strains. Her resistance is complex and very varied, on the one hand by the presence of various chromosomal mutations and on the other hand by the recent emergence of resistance plasmid *mcr*.

Within six months of its discovery, the *mcr1* gene was reported on most continents and ubiquitously present since it has been found in isolates from animals, food, the environment and humans, mcr gene-containing bacteria such as *E. coli*, *Enterobacter*, *Klebsiella, Proteus,Salmonella, Citrobacter, Pseudomonas, Acinetobacter, Kluyvera, Aeromonas, Providencia*, and *Raoultella* have disseminated into environmental reservoirs, including contact surfaces in hospitals, public transportation routes and livestock farms, soil/manure/sludge, plants (vegetables and fruits), aquatic (aquaculture, seawater, ground and surface waters, sewage and wastewaters), and wildlife. These reservoirs are potential sources for further dissemination of *mcr* genes. Anthropogenic activities suchas defecation in open environment/water, bathing/swimming in water bodies, improper disposal ofthe slaughter house, home, hospital and laboratory wastes, inappropriate use of antimicrobial agentsin humans, animals/aquaculture and plants, are the major causes of dissemination of *mcr* genes into the environment. Most resistance to colistin in humans is thought to be due to chromosome resistance.

Phenotypic methods indicate to the microbiologist the presence of polymyxin-resistant strains but do not define the mechanism involved and the risk of transmission. Molecular methods are rapid and more sensitive but are specific to the resistance genes examined and faced with the large number of molecular mechanisms conferring resistance to polymyxins, should only be used to screen *mcr* genes in clinical microbiology laboratories. Genomic analysis enables the complete screening of resistance genes in genetically identified bacteria from clinical samples but remains an *in silico* study which enables predictions but not resistance observation, as the presence of a resistance gene in a genome does not mean that the corresponding isolate is resistant, supported by studies that identified polymyxin-susceptible bacteria carrying the *mcr1* gene. Thus, phenotypic and molecular methods are complementary in detecting colistin-resistant pathogens in order to analyze the behavior of the clinical isolate, and it is important to carry them out in parallel.

Bíblographíc references

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Annexes

Annexe 1

Table 1 :composition of CHROMagar COL-APSE (www.CHROMagar.com)

Product =	Base (B) + Supplement (S)	
Total	42.5 g/L	2 mL/L
Compositon	• Agar 15.0Growthfactorsmix • Peptones 20.0 • Salt 5.0 • Chromogenic and selectve mix 0.8 • Growthfactors 1.7	
Aspect	Powder Form	LiquidForm
STORAGE	15-30 °C 15-30 °C	
FINAL MEDIA pH	7.1 +/- 0.2	

PREPARATION of CHROMagar COL-APSE (Calculaton for 1 L)

Preparaton of Base + S

• Disperse slowly 42.5 g of powder base in 1 L of purifed water.

• Add 2 mL of CHROMagarTM COL-APSE supplement S intoslurry.

• Struntl the agar is well thickened.

• Heat and bring to boiling (100 °C) whiles wirling or strr ngregularly.

Do not heat to more than 100 °c. Do not autoclave at 121 °c

• Cool in a water bath to 45-50 °C, swirling or strringgently to homogenize

Storage :

• Store in the darkbefore use.

• Prepared media plates canbekept for one day at room temperature

• Plates canbestored for up to 1 monthunderrefrigeraton (2/8 °C) if properly prepared and protected from light and dehydraton.

Annexe 2

Preparation of the Rapidpolymyxin NP test :(Bialvaei and al. 2015 ; Humphries and al. 2014)

• To prepare the RapidPolymyxin NP solution, 6.25 g of Mueller Hinton Broth adjusted in cation (MHB-CA) powder, 0.0125 g of phenol red and 225 ml of distilled water were mixed.

•The pH of the solution was adjusted to 6.7

. •The solution was then autoclaved at 121°C for 15 min.

•After cooling the solution to room temperature, 25 ml of D(+)-glucose anhydre 10 % sterilized by filtration, was added.

•Colistin was added extemporaneously to the solution.

•A standardized enterobacterial inoculum was prepared using freshly obtained (overnight) bacterial colonies grown on Luria-Bertani or Mueller Hinton plates.

•The bacterial colonies were resuspended into 10 ml of sterile NaCl 0.85 % to obtain a 3 to 3.5 McFarland optical density (ca. 109 CFU/ml).

• For each isolate, 2 wells are inoculated in parallel with the bacterial suspension, respectively with or without colistin.

• Aftermixing the bacterial suspension to the reactive medium, the final concentration of bacteria was ca. 108 CFU/ml and the final concentration of colistinwas 3.75 µg/ml. The inoculated tray was incubated up to 4h at 35±2°C in ambient air, not sealed and without agitation.

Etude de la résistance à la colistine dans les différents environnements.

Résume

L'objectif de notre travail est d'étudier la prevalence de la résistance à la colistine dans les différents environnements.

La colistine, un antibiotique dit d'ancienne génération dont l'intérêt thérapeutique a été redémontré. En 2015, la toute première résistance plasmidique *mcr-1* a été découverte. Son Développement a été signalé dans nombreuses regions notammenten Europe, en Amériquedu Nord, en Amérique du sud, en Asie et en Afrique du sud dans les isolats bactériens prélevés des humains, des animaux, des fermes, des aliments et de l'environnement. Et son émergence et sa diffusion représentent un problem majeur de santé publique en raison notamment du risqué accru de mortalité. Cette résistance, hautement transférable, reste rare chez l'homme et cela justifie la nécessité de savoir la détecter afin de limiter sa diffusion. Et pour cela, les laboratoires de microbiologie doivent détenir les meilleurs moyens de diagnostiques afin de détecter ces résistances. Il est donc nécessaire d'avoir des techniques de détermination de la sensibilité à la colistine fiables et reproductibles. Et Dans notre travail, nous avons discuté des plus importantes méthodesphénotypiques existantes pour détecter la résistance à la colistine,ainsi que des methods génotypiques pour détecter les mécanismes.

Dans notre etude, nous avons analysé les connaissances actuelles sur l'emergence de la résistance à la colistine dans l'environnement.

Mots clés: colistine, environnement, résistance, détection, *mcr*.

Abstract

The objective of our work is to study the prevalence of resistance to colistin in different environment.

Colistin, a so-called old-generation antibiotic whose therapeutic value has been re-demonstrated,In 2015, the very first *mcr-1* plasmid resistance was discovered. Its development has been reported in many regions including Europe, North America, South America, Asia and South Africa in bacterial isolates collected from humans, animals, farms, food and the environment, and Its emergence and dissemination represent a major public health problemin particular because of the increased risk of mortality. This resistance, highly transferable, remains rare in humans and this justifies the need to know how to detect itin order to limit its distribution. And for this, microbiology laboratories must have the best diagnostic means to detect this resistance. There is therefore a need for reliable and reproducible colistin sensitivity determination techniques. And in our work, we discussed the most important existing phenotypic methods to detect colistin resistance, and also genotypic methods to detect mechanisms.

In our study, we have analysed the current knowledge on the emergence of resistance to colistin.

Key words: colistin, environment, resistance, detection, *mcr*.