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MASTER

Theme

Prevalence of carbapenemase producing GNB isolated in Mustapha Pacha hospital

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Dedications

I dedicate this work first of all to God the Almighty who allowed me to see this day.

To my dear **Dad**, I hope to be your pride as you have always been in my eyes, and may God almighty keep you alive so that you can guide me to other footsteps so serenely and affectionately, no words will be able to express the immense affection and love that I have for you.

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Summary

Tables list:

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List of abbreviations used in the dissertation:

Abbreviation	Term
AMR	Antimicrobial resistance
AK	Amikacin
ATM	Aztreonam
AMP	Ampicillin
AMC	Amoxicillin + clavulanic acid
CTX	Cefotaxime
CAZ	Ceftazidime
COVID-19	Coronavirus disease 2019
CPE	Carbapenemase-producing Enterobacterales
CP-Kp	Carbapenemase-producing *K. pneumoniae*
DD-test	Double Disc test
EDTA	Ethylene diamine tetra acetic acid
ERT	Ertapenem
EUCAST	European Committee on Antimicrobial Susceptibility Testing
ESKAPEE	*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp, Enterobacterales*
ESBL	Extended-spectrum β-lactamase
FOX	Cefoxitine
FEP	Cefepime
GEN	Gentamicin

GNB	Gram-negative bacteria
MEM	Meropenem
MDR	Multidrug resistance
MBLs	Metallo-β-lactamases
Mcr	Mobilized colistin resistance
NDM	New Delhi MBL
ICU	Intensive care unit
IMP	Imipenemase
IMP	Imipenem
KPC	Klebsiella pneumoniae carbapenemase
TOB	Tobramycin
TE	Tetracycline
OXA	Oxacillinases
PAmpC	Plasmidic AmpC
PMQR	Plasmid-mediated quinolone resistance
QNR	Quinolone resistance
VIM	Verona integrin-encoded MBL
WHO	World Health Organization
XDR	Extremely drug-resistant
3GC	3rd Generation Cephalosporin
4GC	4th generation cephalosporin

Introduction

The discovery of penicillin and other antibiotic families and their subsequent large-scale production in the early 20th century was one of the most important achievements in medical history. Antibiotics have saved millions of lives and allowed the advancement of several therapeutics in modern medicine, such as surgery, chemotherapy, etc (Lobanovska and Pilla, 2017). However, their wide use and especially their misuse has led to the emergence of resistant bacterial strains that have subsequently spread throughout the world. This resistance phenomenon affects all classes of antibiotics and has been described worldwide (Irek et al., 2018).

The continued use of antibiotics has created selection pressure that has led bacteria to develop sophisticated resistance mechanisms to escape the activity of these antimicrobial molecules. Several distinct mechanisms can contribute to resistance to a class of antibiotics, and a bacterial cell may use one or more of these resistance mechanisms to escape the effects of an antibiotic (Munita and Arias, 2016). Thus, resistance mechanisms described in bacteria to resist the inhibitory action of antibiotics include (i) production of antibiotic-hydrolyzing or antibiotic-modifying enzymes, such as β-lactamases and aminoglycoside-modifying enzymes; (ii) modification of the target as a result of chromosomal mutations in target genes or by post-translational mechanisms that reduce the affinity of the antibiotic to its target ; (iii) acquisition of an alternative pathway which is insensitive to inhibition by the antibiotic, a mechanism called bypass; (iv) reduced access of the antibiotic to the interior of the bacterial cell, due to reduced permeability of the cell envelope or by active efflux; and (v) production of proteins that protect the target from antibiotic action, such as QNR proteins (Blair et al., 2015; Tenover, 2006; Wright, 2011).

Antimicrobial resistance (AMR) is not a recent phenomenon and many bacteria are naturally resistant to one or more antibiotics. What is new is the speed at which this phenomenon is developing and spreading. This wide dissemination is accelerated by the uncontrolled use of antibiotics in several sectors, not only in human and veterinary medicine but also in animal husbandry. In addition, other factors have contributed to the magnitude of this resistance phenomenon, including the implementation of inappropriate infection control programs, the inability of microbiology laboratories to identify resistant strains, deficiencies in surveillance programs for resistant bacteria, and inadequacies in the regulation of antimicrobial use (Parsonage et al., 2017; Prestinaci et al., 2015). It has been hypothesized that AMR may constitute a leading cause of death worldwide, challenging our ability to treat

infections. For example, it has been estimated that AMR would cause 700,000 preventable human deaths worldwide each year and approximately 10 million deaths per year by 2050 if no action is taken to stop its progression (Tagliabue and Rappuoli, 2018). In addition, Howard and Scott reported that the economic impact related to AMR is expected to cost more than $105 billion per year worldwide (Bush et al., 2011).

Gram-negative bacteria (GNB) form a group of microorganisms most involved in different human infections and infected patients present a high risk of morbidity and mortality (Oliveira and Reygaert, 2021). They are among the most important public health problems in the world due to their continued resistance to antibiotics. Among these GNB, Enterobacterales and Acinetobacter are the most frequently isolated clinical strains in hospitals (Manandhar et al., 2020). They are members of the pathogen group, named ESKAPE, and frequently carry resistance genes to multiple antimicrobial classes, making them multidrug-resistant (MDR) or even extremely drug-resistant (XDR) (Mulani et al., 2019).

Carbapenems are 3-lactam antibiotics and are used as one of the last resorts for the treatment of MDR-GNB infections, including those caused by extended-spectrum 3-lactamase (ESBL) and/or plasmidic AmpC (pAmpC) producing strains. The spread of the latter in the 1990s and 2000s incited clinicians to increasingly rely on the use of carbapenems. Unfortunately, this widespread use has led to the selection of carbapenem-resistant strains in an increasing number of GNB (Kopotsa et al., 2019).

Carbapenem-resistance in GNB is primarily due to the production of carbapenemase enzymes that hydrolyze not only carbapenems but also other 3-lactams (Elshamy and Aboshanab, 2020). Carbapenemase-producing GNB have been listed by the World Health Organization (WHO) as a critical priority of pathogens that pose the greatest threat to human health (World Health Organization, 2017). The three major classes of carbapenemases described worldwide are the Ambler class A carbapenemase of *Klebsiella pneumoniae* (KPC), class B metallo-3-lactamases (MBLs) such as New Delhi MBL (NDM), Verona integrin-encoded MBL (VIM) and imipenemase (IMP), and class D oxacillinase (OXA) enzymes such as OXA-48 carbapenemases in Enterobacteriaceae and OXA-23 in *A. baumannii* (Nordmann et al., 2012).

These carbapenemases have been described worldwide, some of which are typically associated with specific regions or countries (Nordmann et al., 2012). Historically, population movements in border areas, including the movement of refugees and migrants, have played a

key role in the importation and subsequent spread of carbapenemases from endemic to low-endemic areas. Thus, several carbapenemases after their emergence in a given country have been subsequently described worldwide such as KPC in the United States, Greece and Israel, VIM in Greece, OXA-48 in Turkey, and NDM in the Indian subcontinent (van der Bij and Pitout, 2012). The vast majority of these enzymes are localized on transferable plasmids and are associated with a variety of mobile genetic structures (insertion sequences, integrons, and transposons) that promote their spread (Banerjee and Humphries, 2017; Kopotsa et al., 2019).

The spread of carbapenemase-producing GNB is considered a real threat. Thus, high mortality rates have been reported in the wildworld, ranging from 30% to >70%, in infected patients (Sanou et al., 2021). Often, carbapenemase-producing bacteria are also resistant to other families of antibiotics, and many strains are also ESBL-producing or carry plasmid-mediated quinolone resistance (PMQR). These MDR bacteria complicate patient management and limit treatment options (Touati and Mairi, 2020). To address this problem, colistin, as an antibiotic of last resort, has been reintroduced. However, resistant strains and carrying a plasmid transferable resistance "mcr" gene have been reported in recent years in many GNB isolates (Medeiros et al., 2019).

The impact of the continued spread of carbapenemase-producing GNB could impact multiple sectors and complicate infection control in hospitals. The antibiotic resistance phenomenon, including carbapenems resistance, is well documented in Europe, North America, and Asia (Bonomo et al., 2018). However, few studies are available for Africa, especially epidemiological studies conducted in clinical settings. The team of researchers of the unit "Bacterial Pathogenesis and Antibiotic Resistance" directed by Professor A. TOUATI is one of the Algerian team actives in the field of antibiotic resistance in Algeria with several publications on carbapenemases. However, almost all of these publications concern non-hospital strains.

The detection and reporting of carbapenemases in GNB are important to carry out an adapted care policy. Thus, our study aims to report the prevalence and phenotypic characterization of carbapenemase-producing clinical isolates of Enterobacterales (CPE) and *Acinetobacter spp*. at the Mustapha Pacha University Hospital (Algiers, Algeria).

Material and Methods

1. Data collection

This retrospective study was conducted between 15 May and 15 July at the Mustapha Pacha Hospital (1,800 beds) of Algiers, Algeria. The focus was on the carbapenemase producers (Enterobacterales and *Acinetobacter spp.*) among all the samples of inpatients derived from the different hospital wards into the hospital's microbiology laboratory.

Information was collected from each sample file received at the hospital's microbiology laboratory including patient age, ward of hospitalization, specimen source, and date of sampling.

All the biological samples were taken at the level of the different hospital wards by the nursing staff and they were sent to the hospital's microbiology laboratory to be analyzed.

2. Identification

After isolation of strains, bacterial identification was performed using ChromID agar which contains chromogenic substrates allowing the colony staining following degradation by specific bacterial enzymes and the release of the chromophore. Plates were incubated at 37°C for 18 to 24 hours. After incubation, colonies were recognized based on their different color.

After that, two different identification API systems API 20E (Bio-Mérieux, France) and API20NE (Bio-Mérieux, France) were used for the identification of Enterobacterales and *Acinetobacter spp*. Strains respectively.

3. Antimicrobial susceptibility testing

All isolates were tested for antimicrobial susceptibility on Mueller Hinton (Pasteur institute, Algiers) using the disk diffusion method as described by the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2021). Antibiotic discs were deposited on Mueller Hinton agar previously inoculated by swabbing from a bacterial suspension of approximately 10^8 bacteria/mL, and incubated at 37°C/18 to 24 hours. The inhibition zone diameters were measured and interpreted according to the recommendations of EUCAST 2021 **(Table 1)**.

Table 1: Antibiotics tested

Family	Antibiotic	Abbreviation	Disk load (µg)	Critical diameters	
				S≥	R<
Enterobacterales					
f3-lactams	Ampicillin	AMP	10	14	14
	Amoxicillin + clavulanic acid	AMC	20+10	19	19
	Cefotaxime	CTX	5	20	17
	Ceftazidime	CAZ	10	22	19
	Cefoxitine	FOX	30	19	19
	Aztreonam	ATM	30	26	21
	Ertapenem	ERT	10	25	25
	Imipenem	IMP	10	22	19
	Meropenem	MEM	10	22	16
Aminoglycosides	Gentamicin	GEN	10	17	17
	Amikacin	AMK	30	18	18
	Tobramycin	TOB	10	16	16
Fluoroquinolones	Ciprofloxacin	CIP	5	25	22
Tetracyclines	Tetracycline	TE	30	17	17
Acinetobacter spp.					
f3-lactams	Ceftazidime	CAZ	30	18	15
	Imipenem	IMP	10	24	21
	Meropenem	MEM	10	21	15
Aminoglycosides	Gentamicin	GEN	10	17	17
	Amikacin	AMK	30	19	19
	Tobramycin	TOB	10	17	17
Fluoroquinolones	Ciprofloxacin	CIP	5	50	21
Tetracyclines	Tetracycline	TE	30	15	12

4. Detection of carbapenemase:

4.1. Hodge test:

The production of a carbapenemase was investigated in strains that showed a decrease in their susceptibility to carbapenems. An imipenem disc was deposited in the center of a Mac Conkey plate previously inoculated with *E. coli* strain ATCC 25922 (susceptible to all antibiotics). After that, the strain to be tested, a positive control (*K. pneumoniae* NDM-5) and a negative control (*E. coli* ATCC 25922) were inoculated on the agar in the form of streaks deposited from the imipenem disc to the periphery of the Mac Conkey plate. After 18-24 hours

of incubation at 37°C, the production of a carbapenemase results in a distortion of the zone of inhibition around the imipenem disc (Lee et al., 2010).

4.2. Detection of Metallo-β-lactamases (MβL):

a) Combined disc method:

After inoculated a Mueller Hinton agar with the strain to be tested, two imipenem discs (IMP, 10tg) and a blank disc were placed separately. A volume of 10 tl of an EDTA solution (0.5 M, pH 8) was added to one of the imipenem discs in addition to the blank disc used as a witness. After incubation at 37°C/18 hours, strains with an inhibition diameter around the disc IMP-EDTA is greater than that obtained with the IMP disc alone, at least 6 mm, are considered to be MβL producing strains (Yong et al., 2002).

b) Synergy by the DD-test:

This test consists of depositing an imipenem disc (IMP, 10tg) at a distance 15mm of a blank disc soaked in 10µl of EDTA solution (0.5 M, pH 8). The presence of an MβL was detected by appearance a synergy image between the two discs (Jeong et al., 2006).

4.3. Detection of classe A carbapenemase:

The presence of a classe A carbapenemase was detected using combined disc method which consisted of deposing two imipenem discs (IMP, 10tg). A volume of 10 tl of boronic acid was added to one of the imipenem discs. After incubation at 37°C/18 hours, strains with an inhibition diameter around the disc IMP-boronic acid is greater than that obtained with the IMP disc alone, at least 6 mm, are considered to be classe A cabapenemase producing strains.

5. Detection of Extended Spectrum β-Lactamases (ESBLs) for CPE strains:

The presence of an ESBL was detected using Double Disc synergy test (DD-test) which consisted of depositing discs of ceftazidime (CAZ, 30 tg), cefotaxime (CTX, 30 tg), aztreonam (ATM, 30 tg) and cefepime (FEP, 30tg) at a distance of 20 mm (center to center) from an amoxicillin-clavulanic acid disc (AMC, 20/10 tg). The appearance of a synergy between the amoxicillin-clavulanic acid disc and the ceftazidime, cefotaxime, aztreonam and cefepime discs indicates the production of an ESBL (Jarlier et al., 1988) (Jarlier et al., 1988).

Results

1. Demographic and clinical characteristics

During the study period, a total of 12 bacterial isolates (04 Enterobacterales and 08 *Acinetobacter baumannii*) with reduced susceptibility to carbapenems were collected from1106 samples, giving an overall prevalence of 1.08% (Table 2).

The four Enterobacterales isolates were identified as *Klebsiella pneumoniae* (n=3/4), *Enterobacter cloacae* (n=1/4).

The demographic and clinical characteristics of the 12 patients are summarized in **Table 2**. Of them, 7 (58.3%) were male and 9 (75%) were adults. The patients were admitted in different hospital wards including intensive care unit (n=6; 50%), diabetology (n=2; 16.6%), orthopedic (n=1; 8.3%), pediatric (n=1; 8.3%), surgery (n=1; 8.3%), and pediatric surgery (n=1; 8.3%).

The bacterial strains were isolated from wound (n=4; 33.3%), blood (n=4; 33.3%), bronchial (n=1; 8.3%), urine (n=1; 8.3%), peritoneal fluid (n=1; 8.3%), and central catheter (n=1; 7.1%).

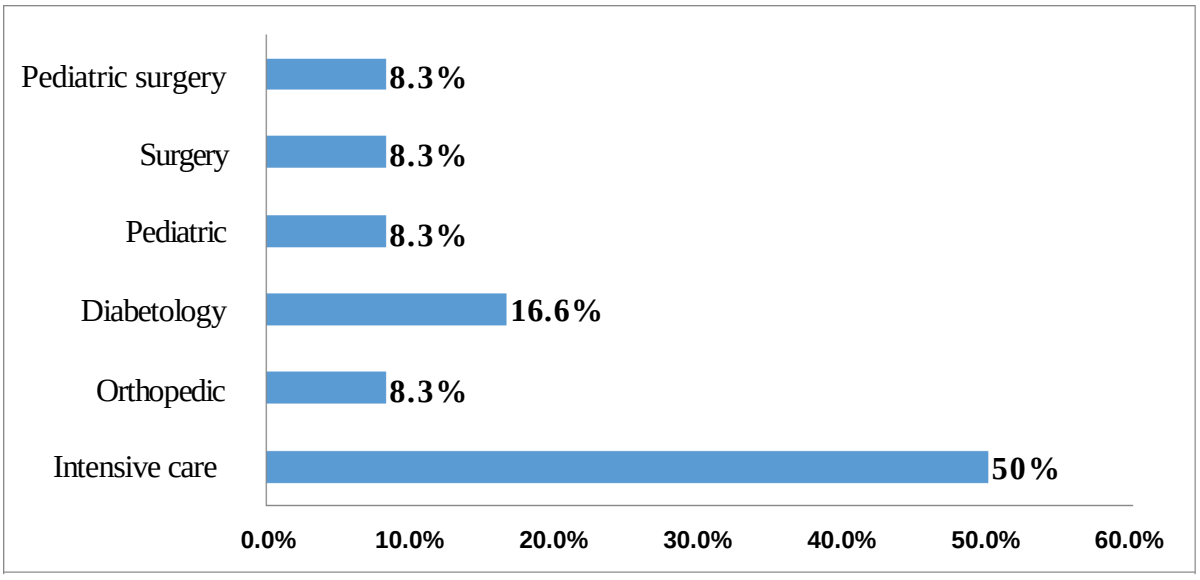


Table 2: Demographic and clinical characteristics of the patients

Patient	Strain	Specimen source	Date of sampling	Gender	Age	Hospital ward
Patient 1	727	Peritoneal fluid	24/06/2021	Male	Child	Pediatric surgery
Patient 2	792	Blood	28/06/2021	Male	Child	Intensive care unite
Patient 3	804	Urine	28/06/2021	Male	Adult	Intensive care unite
Patient 4	847 I	Blood	03/07/2021	Female	Child	Pediatric
Patient 5	16	Central catheter	25/06/2021	Female	Adult	Intensive care unite
Patient 6	122	Bronchial	02/06/2021	Female	Adult	Intensive care unite
Patient 7	294	Wound	01/06/2021	Male	Adult	Diabetology
Patient 8	310	Wound	30/05/2021	Female	Adult	Orthopedic
Patient 9	409	Wound	27/06/2021	Male	Adult	Diabetology
Patient 10	572	Wound	03/06/2021	Male	Adult	Surgery
Patient 11	768	Blood	25/06/2021	Female	Adult	Intensive care unite
Patient 12	786A	Blood	01/07/2021	Male	Adult	Intensive care unite

2. Susceptibility to antibiotics

The results of antibiotics susceptibility of the Enterobacterales and *A. baumannii* strains to β-lactams and aminoglycosides are shown in **Table 3** and **Table 4**, respectively.

All Enterobacterales isolates were resistant to β-lactams including ampicillin and amoxicillin-clavulanic acid, cefotaxime, ceftazidime, ertapenem, and meropenem (n=100%), followed by resistance to cefepime (n=3/4), and imipenem (n=2/4), and cefoxitin (n=1/4). In addition, Enterobacterales isolates showed resistance to non β-lactams antibiotics including ciprofloxacin (n=3/4), gentamicin (n=2/4), amikacin (n=2/4), and tobramycin (n=2/4). All strains were resistant to tetracycline. **(Table 3 and Figure 1).**

Table 3: Antimicrobial profiles of the Enterobacterales isolates

Patient	Strain	Species	AMR profiles													
			AMP	**AMC**	**CTX**	**CAZ**	**FOX**	**FEP**	**ERT**	**MEM**	**IMP**	**CIP**	**TOB**	**AMK**	**GEN**	**TE**
Patient1	**727**	*E. cloacae*	<06(R)	<06(R)	<06(R)	<06(R)	<06(R)	28(S)	17(R)	20(I)	24(S)	23(I)	17(S)	24(S)	16(R)	<06(R)
Patient2	**792**	*K. pneumoniae*	<06(R)	<06(R)	<06(R)	<06(R)	25(S)	11(R)	<06(R)	<06(R)	<06(R)	8(R)	<06(R)	17(R)	<06(R)	<06(R)
Patient3	**804**	*K. pneumoniae*	<06(R)	<06(R)	<06(R)	<06(R)	28(S)	<06(R)	21(R)	21(I)	21(I)	11(R)	9(R)	17(R)	8(R)	10(R)
Patient4	**847I**	*K. pneumoniae*	<06(R)	<06(R)	16(R)	12(R)	26(S)	22(R)	15(R)	19(I)	30(S)	25(S)	20(S)	19(S)	19(S)	12(R)

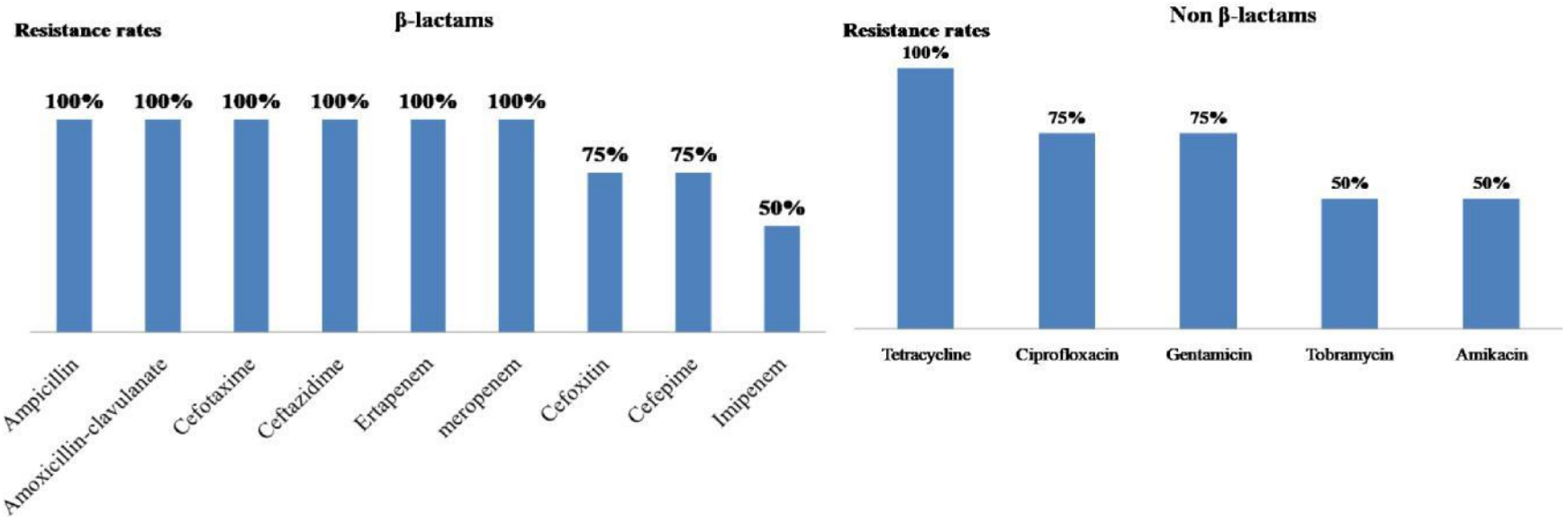


Figure 1: Resistance rates of Enterobacterales strains to antibiotics

All *A. baumannii* isolates were resistant to ceftazidime, imipenem, meropenem, amikacin, gentamicin, and tertacycline (n=8; 100%), followed by tobramycin (n=6; 75%) **(Table 4 and Figure 2)**.

Table 4: Antimicrobial profiles of *A. baumannii* isolates

Patient	Strain	AMR profiles							
		CAZ	IMP	MEM	CIP	GEN	TOB	AMK	TE
Patient 5	**16**	<06 (R)	<06 (R)	<06 (R)	<06 (R)	<06 (R)	<06 (R)	<06 (R)	12 (R)
Patient 6	**122**	<06 (R)	<06 (R)	<06 (R)	9 (R)	10 (R)	11 (R)	<06 (R)	<06 (R)
Patient 7	**294**	<06 (R)	<06 (R)	<06 (R)	9 (R)	15 (R)	20 (S)	<06 (R)	<06 (R)
Patient 8	**310**	<06 (R)	<06 (R)	<06 (R)	11 (R)	<06 (R)	10 (R)	12 (R)	15 (R)
Patient 9	**409**	<06 (R)	<06 (R)	<06 (R)	8 (R)	14 (R)	19 (S)	<06 (R)	<06 (R)
Patient 10	**572**	<06 (R)	<06 (R)	<06 (R)	<06 (R)	<06 (R)	<06 (R)	<06 (R)	<06 (R)
Patient 11	**768**	<06 (R)	<06 (R)	<06 (R)	<06 (R)	<06 (R)	<06 (R)	<06 (R)	12 (R)
Patient 12	**786A**	<06 (R)	<06 (R)	<06 (R)	10 (R)	<06 (R)	<06 (R)	<06 (R)	17 (R)

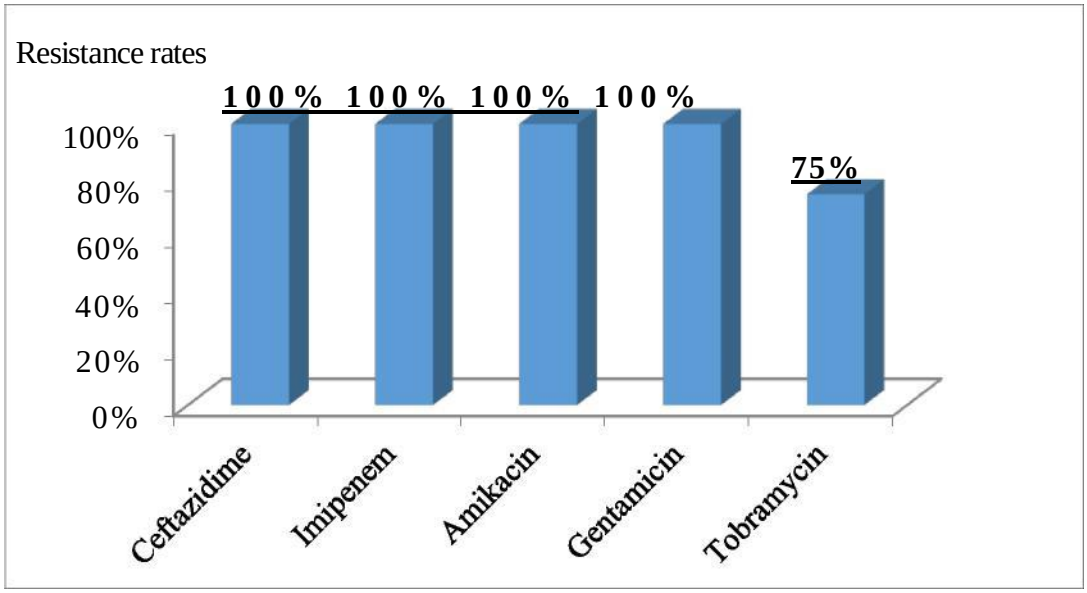
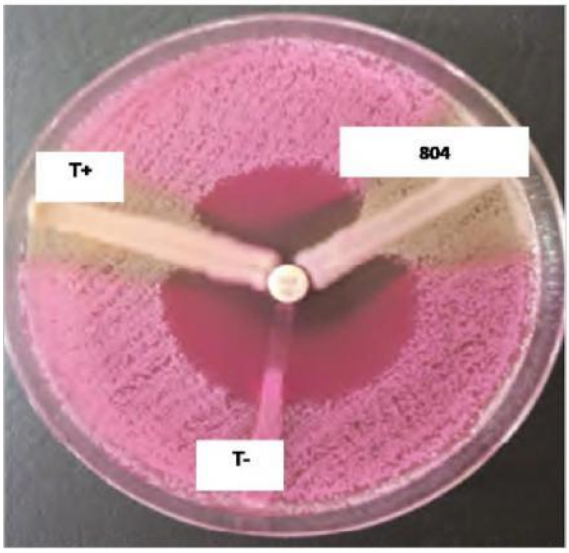


Figure 2: Resistance rates of *A. baumannii* strains to antibiotics

3. Analysis of resistance phenotypes

The Hodge test was positive for all strains resistant to ertapenem and meropenem indicating the probable production of a carbapenemase **(Figure 3)**. No strain was inhibited by EDTA indicating absence of MβL. One CPE strain (792) was inhibited by boronic acid indicating the presence of classe A carbapenemase **(Table 5 and Figure 4)**. All *A. baumannii* produced oxacillinase-type carbapenemases



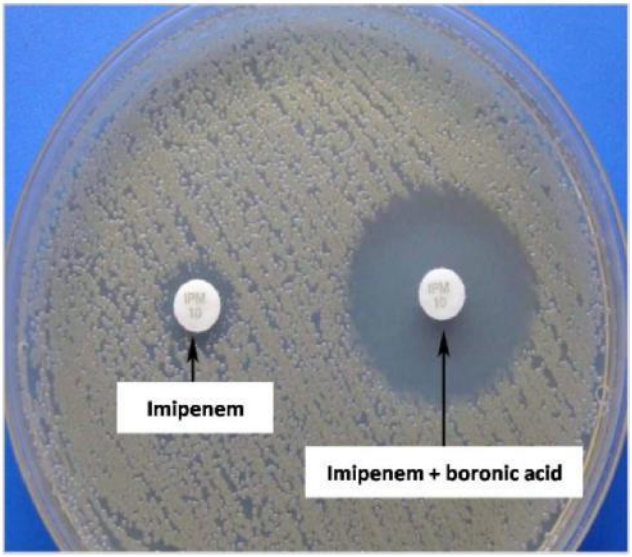


Figure 3: Hodge test positive for 804 strain **Figure 4:** Boronic acid positive for 792 strain

The DD-test performed on Mueller Hinton agar showed a synergy picture in two CPE strains and were resistant to 3GC and 4GC reflecting the probable production of ESBL **(Table 4 and Figure 5)**.

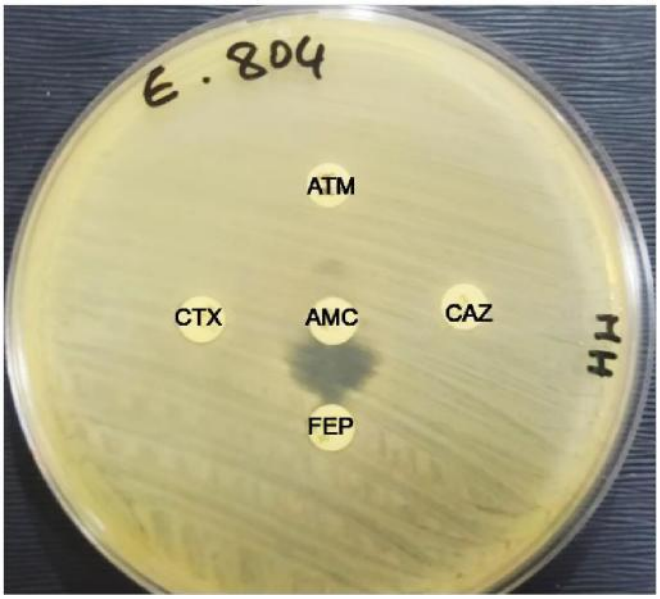


Figure 5: DD-test positive for 804 strain

Table 5: Resistance phenotypes to β-lactams to *A.baumannii* strains isolated from patients

Patient	Strain	Species	CAZ	MEM	IMP	EDTA	Boronic Acid	Phenotype
Patient 5	**16**	*A. baumannii*	R	R	R	-	-	**Oxacillinase-type**
Patient 6	**122**	*A. baumannii*	R	R	R	-	-	**Oxacillinase-type**
Patient 7	**294**	*A. baumannii*	R	R	R	-	-	**Oxacillinase-type**
Patient 8	**310**	*A. baumannii*	R	R	R	-	-	**Oxacillinase-type**
Patient 9	**409**	*A. baumannii*	R	R	R	-	-	**Oxacillinase-type**
Patient 10	**572**	*A. baumannii*	R	R	R	-	-	**Oxacillinase-type**
Patient 11	**768**	*A. baumannii*	R	R	R	-	-	**Oxacillinase-type**
Patient 12	**786A**	*A. baumannii*	R	R	R	-	-	**Oxacillinase-type**

Table 5: Resistance phenotypes to β-lactams to Enterobacterales strains isolated from patients

Patient	Strain	Species	AMC	CTX	CAZ	FOX	ERT	MEM	IMP	DD-test	EDTA	Boronic Acid	Phenotype
Patient 1	**727**	*E. cloacae*	R	R	R	R	R	I	S	-	-	-	OXA-48-like
Patient 2	**792**	*K. pneumoniae*	R	R	R	S	R	R	R	-	-	+	Classe A carbapenemase
Patient 3	**804**	*K. pneumoniae*	R	R	R	S	R	I	I	+	-	-	OXA-48-like+ESBL
Patient 4	**847 I**	*K. pneumoniae*	R	R	R	S	R	I	S	+	-	-	OXA-48-like+ESBL

Disscussion

The continuous increase of antibiotic resistance is a major public health problem and poses a real challenge in the therapeutic choices for the treatment of infections. The rates of carbapenem resistance among GNB isolates have reached alarming proportions in recent years, thus limiting the number of molecules that can be used for the treatment of infections due to multidrug-resistant GNB. In Algeria, the majority of available publications have reported carbapenem-resistant Enterobacteriaceae isolates in non-hospital niches and few studies have reported these strains in clinical samples. This is not the case for *A. baumannii* strains, where the majority of articles have reported strains isolated from hospitalized patients (Bourafa et al., 2018).

Contrary to several neighboring countries, the epidemiology of clinical strains of CPE and *Acinetobacter spp*. has not been well characterized in Algeria. We identified 14 articles and 8 articles reporting CPE and *Acinetobacter spp*. in Algeria in the PubMed database, respectively. The report prepared by WHO experts emphasized the need for an integrated surveillance program and the implementation of a very active investigation to highlight the extent of resistant strains in developing countries (Eshetie et al., 2015).

In the present study, we estimated the overall prevalence of carbapenemase producers at Mustapha Pacha Hospital to 1.08%. In a study conducted at Constantine hospital, Agabou et al. and Leulmi et al. reported a prevalence of 0.22% (1/448) and 1% (1/97) in different clinical strains, respectively (Agabou et al., 2014; Leulmi et al., 2019). In Annaba hospital, an increase in the prevalence of carbapenemase-producing strains was recorded from 2.9% (3/105) to 10.4% (5/47), respectively in 2014 and 2018 (Bourafa et al., 2018; Sassi et al., 2014). Recently, a prevalence of 0.5% was reported by Nabti et al. at Setif hospital among urinary *E. coli* strains (Nabti et al., 2019).

In our study, carbapenemase producers were obtained from patients admitted to different hospital wards, nevertheless, the strains were mostly obtained from the intensive care unit (50% of the strains). It is widely reported that patients admitted to intensive care units (ICU) are at higher risk of contracting nosocomial infections, due to their underlying diseases or weakened immunity (Raro et al., 2017).

Strains of *A. baumannii* are known to be one of the most antimicrobial-resistant and difficult to control members of the GNB. These strains have been implicated in various infections such as bacteremia, pneumonia, urinary tract and wound infections (Amiri et al.,

2017). In our study, *A. baumannii* strains were mainly isolated from surgical wounds. Historically, it has been associated with opportunistic infections; however, in the last two decades, there has been an increase in the incidence and severity of *A. baumannii* infections. Known risk factors for acquiring *A. baumannii* infections generally include the length of stay in intensive care units, use of central venous catheters, and use of antibiotics (Amiri et al., 2017).

In this study, *A. baumannii* strains are not only resistant to beta-lactams but are also resistant to amikacin, gentamicin, and tetracycline. These results are quite similar to those reported in the literature (Kindu et al., 2020).

Carbapenem resistance in *A. baumannii* is mainly related to the production of carbapenemases, mostly oxacillinases, including OXA-23, OXA-24/40, and OXA-58 (Raro et al., 2017). In our study, the phenotypic tests performed are in favor of concluding that *A. baumannii* strains are probably oxacillinase-producers.

Recently, we have witnessed a dramatic increase in the prevalence of infections caused by CPE strains, which has a major impact on public health. The most frequently encountered carbapenemases are KPC, NDM, and OXA-48. The first case of OXA-48 in Algeria was reported in a clinical *E. coli* isolate by Agabou et al. at Constantine Hospital (Touati and Mairi, 2020). This carbapenemase has become endemic in North Africa. These enzymes are frequently found on mobile genetic elements and have the potential to be widespread worldwide (Touati and Mairi, 2020).

In our study, *K. pneumoniae* (n=3/4) was the major carbapenemase-producing species among the Enterobacterales studied. Similarly, in their recently published systematic review, Touati and Mairi reported that among the 79 clinical CPE recorded, 78.5% (n=62) of these strains were *K. pneumoniae* (Touati and Mairi, 2020).

In addition to f3-lactam resistance, a relatively high percentage of resistance to non-f3-lactam antibiotics including; ciprofloxacin, gentamicin, amikacin, and tobramycin was also identified. This multidrug resistance may complicate the treatment of infections due to these MDR strains. In their study, Loucif et al. reported a nosocomial outbreak of OXA-48 producing *K. pneumoniae* in Batna hospital. During this epidemic episode, five patients in the hematology unit infected with an ertapenem-resistant strain of *K. pneumoniae* died after several combination therapies (cefotaxime+ gentamicin + metronidazole, amikacin + piperacillin, imipenem+ vancomycin + ofloxacin) (Loucif et al., 2016). The use of colistin for the treatment

of carbapenemase-producing GBN infections is not without risk due to the recent emergence of colistin resistance that is either plasmid (MCR production) or chromosomal (mutations in different operons involved in LPS synthesis) (Battikh et al., 2017; Mansour et al., 2017) (Battikh et al., 2017; Mansour et al., 2017)

Several studies have reported the simultaneous production of ESBLs in association with carbapenemases in Enterobacterales strains has been reported. The blaCTX-M type ESBLs were the most frequently detected. Thus, Touati and Mairi reported in their systematic review that out of 191 OXA-48-like CPE strains, 32 strains (16.8%) co-produced the ESBL CTX-M-15 alone or in association with other β-lactamases (TEM-like and SHV-12). They noted that the frequency of strains producing the OXA-48+CTX-M-15 combination was higher in clinical strains (n=22/67; 32.8%) than in extra-human strains (n=8/123; 6.5%) (Touati and Mairi, 2020).

Phenotypic detection of carbapenemase-producing bacteria is not always easy because no commercially available screening medium can detect all types of carbapenemases. In addition, some carbapenemase-producing strains show only intermediate resistance to carbapenems in routine testing, making their detection difficult (Manenzhe et al., 2015). In this study, phenotypic tests were performed to detect the type of carbapenemases produced by the isolates. Thus, we believe that Enterobacterales strains are probably producers of two types of carbapenemases including either an OXA-48 type carbapenemase associated or not with an ESBL or the production of a class A carbapenemase, probably a KPC, in one strain.

The sensitivity and specificity of phenotypic tests are not always conclusive. Molecular methods are currently favored because most of them can be performed rapidly with a high level of accuracy and in a very short period. Molecular characterization of antibiotic resistance mechanisms and molecular epidemiology of resistant strains represent a crucial step to fight against its spread and to develop therapeutic strategies (Bedenić, 2019). However, in Algeria, almost all laboratories do not have molecular methods which are a major handicap for the management of infections due to carbapenemase-producing strains. Thus, the implementation of molecular methods in the routine of microbiology laboratories is more important than ever, especially in this period of the COVID19 pandemic.

This short study led to interesting results with certain practical learning. Thus, during this practical course, we learned a set of bacteriological methods and we acquired some know-how in the field of bacteriology. However, the results obtained have several limitations. On the one hand, the small number of carbapenemase strains collected may limit the generalization of

the results, and on the other hand, it was carried out during a relatively short period and under restrictive handling conditions due to the exceptional sanitary context we are currently experiencing caused by the worldwide pandemic of COVID-19. It is worth mentioning that during our practical training, we collected all ESKAPEE pathogens. However, with the confinement and restrictions we underwent, we could not characterize all the strains collected in the laboratory and we opted only for the characterization of carbapenemase-producing strains.

In Algeria, the prevalence of MDR is probably underestimated because Algerian clinical laboratories have only phenotypic methods based essentially on the interpretation of the results of susceptibility testing of strains determined by the disc diffusion method on Mueller Hinton agar. This situation could increase the risk of epidemics in Algeria because the strains are not genetically characterized. In the different publications made on this theme, the molecular analysis of strains has been made possible by a large network of collaborations between Algerian university laboratories and French clinical microbiology laboratories. Thus, the published work of Ph.D. students has been of great help in the surveillance of antibiotic resistance in Algeria.

Conclusion

The work developed in the framework of this master dissertation has a multidisciplinary character. In addition to its academic character allowing to realize a master dissertation, the subject treated is in direct relation with the concerns of the hospitals and thus of the health sector. To complete this modest work, it was necessary to take several individual steps towards several health structures, because we found that there were no cooperation agreements between our university and the various university hospitals we contacted. Thus, it would be judicious to conclude cooperation agreements between the various sectors on which the various collaborative actions will be based, in particular those related to sensitive research and current affairs themes, such as our master's thesis subject. Such cooperative actions on topics of common interest will have positive repercussions on both sectors and will also allow for the development of interesting human and professional relationships, in addition to the exchange of ideas and the capital of experience between both parties.

This study on carbapenem-resistant bacteria opens several prospects:

- **Longer surveillance with larger sample size is needed to better understand the epidemiology and spread of these bacteria in our hospital facility,**
- **The study must be completed with the implementation of molecular methods for genetic investigations of multi-resistant strains, to enrich the study with several details to understand the different resistance mechanisms and especially to know the origin of these strains.**

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Abstract

Background: Carbapenemase-producing Gram negative bacteria are emerging rapidly causing global epidemic infections. These infections are associated with high costs and cause major difficulties of treatment due to a limited therapeutic alternative. However, the detection of carbapenemases is required to infection control. The aim of the current study was to characterize the prevalence of the carbapenemase-producers among Enterobacterales and *Acinetobacter spp*

Methods: This retrospective study was conducted during 2 months between 15 May and 15 July at the Mustapha Pacha Hospital of Algiers, Algeria. Clinical data were collected on all included patients; the bacterial identification was performed using ChromID agar, and confirmed by API systems. Antimicrobial susceptibility was tested for all isolates on Mueller Hinton using the disk diffusion method. The carbapenemases were detected by Hodge test, phenotypic tests were carried out for the detection of the type of carbapenemases. The ESBLs were detected by DD-test.

Results: A total of 12 carbapenemase strains were isolated from 12 patients, ncluding 04 Enterobacterales and 08 *Acinetobacter baumannii* strains. Patients were 58.3% male and 75% were adults, they were admitted in different hospital wards; mostly from intensive care ward (50%). The bacterial strains were mostly obtained from wound and blood (33.3%). *Klebsiella pneumoniae* was the main isolated bacteria, All Enterobacterales isolates showed resistance to f3-lactams and non f3-lactams. All *A. baumannii* isolates were resistant to ceftazidime, imipenem, meropenem, amikacin, gentamicin, and tertacycline (n=8; 100%). Hodge test was positive for all strains resistant to ertapenem and meropenem indicating the probable production of a carbapenemase. No strain was inhibited by EDTA indicating absence of Mf3L. One Enterobacterale strain was inhibited by boronic acid indicating the presence of classe A carbapenemase. All *A. baumannii* produced oxacillinase-types carbapenemases.

Conclusion: This study revealed the presence and the diversity of clinically relevant carbapenemase bacteria at Mustapha Pasha Hospital in Algiers.

Keywords: Hospital, Gram negative bacteria, Carbapenemases.

Résumé

Les bactéries à Gram négatives productrices de carbapénémases émergent rapidement, provoquant des épidémies mondiales. Ces infections sont associées à des coûts élevés et entraînent des difficultés majeures de traitement en raison d'une alternative thérapeutique limitée. Par conséquent, le but de la présente étude était de caractériser la prévalence des bactéries productrices de carbapénémases chez les Entérobactéries et *Acinetobacter spp*.

Méthodes : Cette étude rétrospective a été menée pendant 2 mois entre le 15 mai et le 15 Juillet à l'hôpital Mustapha Pacha d'Alger en Algérie. Les données cliniques ont été recueillies sur tous les patients ; l'identification bactérienne a été réalisée en utilisant la gélose ChromID, puis deux systèmes API d'identification différents (API 20E et API20NE). La sensibilité aux antibiotiques a été testée pour tous les isolats sur Mueller Hinton en utilisant la méthode de diffusion sur disque. Ensuite la détection des carbapénémases par test de Hodge, enfin des tests phénotypiques ont étaient réalisés pour la détection du type des carbapenamses.

Résultats : Au total de 12 souches carbapénémases ont été isolées chez 12 patients, dont 04 souches d'Enterobactéries et 08 souches *d'Acinetobacter baumannii.* Les patients étaient 58,3 % de sexe masculin et 75 % adultes, ils ont été admis dans différents services hospitaliers ; principalement de la salle de soins intensifs (50%). Les souches bactériennes ont été principalement obtenues à partir de plaies et de sang (33,3 %). *Klebsiella pneumoniae* était la principale bactérie isolée. Tous les isolats d'Enterobactéries ont montré une résistance aux -lactamines et aux non f3-lactamines. Tous les isolats d'*A. baumannii* étaient résistants à la ceftazidime, à l'imipénème, au méropénème, à l'amikacine, à la gentamicine et à la tertacycline (n = 8 ; 100 %). Le test de Hodge était positif pour toutes les souches résistantes à l'ertapénème et au méropénème indiquant la production probable d'une carbapénémase. Aucune souche n'a été inhibée par l'EDTA indiquant l'absence de Mf3L. Une souche d'entérobactérie a été inhibée par l'acide boronique, indiquant la présence de carbapénémase de classe A. Tous les *A. baumannii* ont produit des carbapénémases de type oxacillinase

Conclusion : Cette étude a révélé la présence et la diversité de bactéries carbapénémases cliniquement pertinentes résistantes aux antibiotiques à l'hôpital Mustapha Pacha d'Alger.

Mots cles: Hopital, bacteries Gram negatives, Carbapenemases.