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RESEARCH ARTICLE

Characterization of imipenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* clinical isolates in a Moroccan hospital

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ABSTRACT

Acinetobacter baumannii and *Pseudomonas aeruginosa* are two pathogens with an important power of adaptation to antibiotics thus, both pose a real public health problem. Our study investigated epidemiological characteristics, antibiotic sensitivity profile and resistance genes of imipenem resistant *A. baumannii* and *P. aeruginosa*. This was a retrospective study carried out in the bacteriology laboratory of Mohammed V military training hospital, spanning from January 2018 to April 2021. Antibiotic susceptibility was studied by Mueller Hilton agar diffusion method with OXOID® type antibiotic discs and interpreted according to the recommendations of EUCAST 2021. Carbapenemase detection was performed by CarbaNP-test®. The molecular study was performed using conventional PCR. During the study period, we collected 1,072 imipenem-resistant isolates namely, 820 *A. baumannii* and 252 *P. aeruginosa*. The molecular study showed that out of 108 *A. baumannii* isolates 102 carried the $bla_{\text{OXA-51}}$ and 100 isolates carried the $bla_{\text{OXA-23}}$ gene. The coexistence of $bla_{\text{OXA-23}}$ and bla_{NDM} genes was detected in only 4 isolates. Altogether 50% of *P. aeruginosa* strains carried $bla_{\text{VIM-2}}$. All investigated *A. baumannii* and *P. aeruginosa* strains were colistin susceptible in this study. Multiresistant bacterial infections are associated with longer hospitalization, higher hospital costs and higher mortality rates. Therefore, a collective action including the different actors of the healthcare system is necessary.

KEYWORDS

Acinetobacter baumannii, Pseudomonas aeruginosa, OXA-23, NDM-1, VIM-2, PCR

INTRODUCTION

Gram-negative non-fermenting bacilli are widely disseminated in the hospital environment and are highly associated with serious healthcare-related infections, especially in immunocompromised patients [1, 2]. Gram-negative non-fermenting bacilli are resistant to most antibiotics, mainly due to their ability to acquire resistance genes by horizontal transfer, but also because of their ability to survive in the environment for long periods [3]. The infections caused by these multi-resistant bacteria are characterized by their opportunistic nature and their cumbersome and costly management as well as their high morbidity and mortality

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rate [4]. These infections are frequently found in intensive care patients due to mechanical ventilation, the use of the central venous line and urinary catheters [5].

Gram-negative non-fermenting bacilli can exhibit different mechanisms of intrinsic and acquired resistance to a large number of antibiotics commonly used in medical therapy, such as penicillins, cephalosporins, aminoglycosides, and fluoroquinolones. Carbapenems are still the drugs of choice for treating infections associated with these multi-resistant bacteria [2]. However, resistance to carbapenems in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* led to increased use of last resort antibiotics (polymyxin B and tigecycline) to treat this kind of infections [2].

Among resistance mechanisms of these bacteria, the major mechanism is production of different carbapenemases that hydrolyse the antibiotics on their β-lactam cycle. These enzymes are divided into different classes according to Ambler's classification [6]. The production of these enzymes results from the expression of different genes namely, *Klebsiella pneumoniae* carbapenemase (KPC), Guiana extended spectrum (GES) in Ambler class A, Imipenemase (IMP), Verona integron-encoded metallo-β-lactamase (VIM), New Delhi metallo-β-lactamase (NDM), São Paulo metalo-beta-lactamase (SPM), German imipenemase (GIM) in Ambler class B while Oxacillinase (OXA) in Ambler class D [7].

Our study investigated epidemiological characteristics, antibiotic sensitivity profile and resistance genes of imipenem resistant *A. baumannii* and *P. aeruginosa*.

METHODS

Study design, patients, and bacteria collection

This was a retrospective study carried out in the medical bacteriology laboratory of Mohammed V military training hospital in Rabat, spanning 40 months from January 2018 to April 2021. All isolates of *A. baumannii* and *P. aeruginosa* resistant to imipenem from different types of clinical samples were included.

Identification of bacterial isolates was based on culture, morphological and biochemical identification characteristics. Biochemical identification was performed by API20NE ready-to-use galleries (bio-Mérieux SA, Marcy-l'Étoile/France).

Antimicrobial susceptibility testing

Antibiotic susceptibility was studied by Mueller-Hilton agar diffusion method with OXOID® type antibiotic discs and interpreted according to the recommendations of EUCAST 2021 [8]. The interpretation was performed using the Adagio Biorad® system. Quality control of the antibiotic susceptibility test was performed with the *P. aeruginosa* strain ATCC 27853. For colistin, determination of the minimum inhibitory concentration was performed by microdilution method using the sensititre COL* plate (Thermo Scientific™, France) from an 18–24-h culture. Interpretation of results was performed according to EUCAST 2021 recommendations [8]. Carbapenemase detection was performed by Carba-test®. This test is based on a color change of a chromogenic substrate in the presence of a carbapenemase-producing strain. Testing was conducted directly in microtubes with freshly isolated Enterobacteriaceae colonies and the reading should be taken within 30 min [9].

Screening of bla_{OXA}, bla_{VIM}, bla_{IMP} and bla_{NDM} genes among imipenem-resistant isolates

Among the 1,072 isolates resistant to imipenem, we randomly selected 108 isolates of *A. baumannii* and 16 isolates of *P. aeruginosa*. These isolates were studied phenotypically using the CarbaNP test and genotypically. DNA extraction was performed by heat shock from overnight cultures. The DNA extracts were quantified by NanoDrop (Thermo Fisher Scientific, Massachusetts, USA) and stored at −20 °C, to be used as templates in PCRs. We performed two multiplex PCRs to detect the eight genes encoding carbapenemases. The first multiplex PCR targeted three genes coding for class D carbapenemases (bla_{OXA-51}, bla_{OXA-23}, bla_{OXA-24}) [10] and one gene from the class B metallo-beta-lactamases (bla_{IMP}) [10]. The second multiplex PCR included one class D gene (bla_{OXA-23}) [14] and three class B genes (bla_{IMP}, bla_{VIM-2}, bla_{NDM}) [11–13].

PCR amplification of the studied genes was performed in a final volume of 25 µl, the reaction mixtures contained 5 µl of buffer, 0.5 µl of Taq DNA polymerase, and 0.5 µl of each primer and reaction mix was completed with water until a volume of 22 µL, and finally 3 µl of DNA template was added to the reaction. Amplification conditions using MyCycler™ thermal cycler (BioRad, Germany) were initial denaturation at 95 °C for 1 min, followed by 35 cycles of 95 °C for 15 s, and annealing (56 °C for 15 s for multiplex 1 and 54 °C for 15 s for multiplex 2), with a final extension for 10 s at 72 °C [10–14]. The melting temperatures are shown in Tables 1 and 2. The expected amplicons were detected by 1% agarose gel electrophoresis containing ethidium bromide for 90 min at 100 V, and the DNA bands were visualized by ultraviolet (UV) light. Data extraction was performed using the epidemiological model of the Adagio Biorad® antibiotic susceptibility testing system and the Laboratory Information System (LIS). Duplicates were excluded.

Moroccan legislation does not require ethical approval for retrospective studies based on laboratory data.

Statistical analysis

The data entry was done on Excel and the statistical analysis on SPSS (Statistical Package for Social Sciences) 20.0. This analysis included a descriptive part of all the collected data, the qualitative variables were expressed in numbers and percentages.

RESULTS

During the study period, we collected 1,072 isolates of imipenem-resistant *A. baumannii* and *P. aeruginosa*, of which

Table 1. Primers used for amplification of carbapenemase genes. (Multiplex 1)

Primers	Nucleotide sequences (5→3)	Amplicon size (bp)	Melting temperature (°C)	References
IMP F	AACCAGTTTTGCCTTACCAT	587	56	[10]
IMP R	CTACCGCAGCAGAGTCTTTAC			
OXA-51 F	TAATGCTTTGATCGGCCTTG	353	56	[10]
OXA-51 R	TGGATTGCACTTCATCTTGG			
OXA-23 F	GATCGGATTGGAGAACCAGA	501	56	[10]
OXA-23 R	ATTTCTGACCGCATTTCCAT			
OXA-24 F	TTCCCCTAACATGAATTTGT	1,024	56	[10]
OXA-24 R	GTACTAATCAAAGTTGTGAA			

Table 2. Primers used for amplification of carbapenemase genes. (Multiplex 2)

Primers	Nucleotide sequences (5→3)	Amplicon size (bp)	Melting temperature (°C)	References
NDM F	GGTTTGGCGATCTGGTTTTC	621	54	[11]
NDM R	CGGAATGGCTCATCACGATC			
VIM-2 F	ATGTTCAAACTTTTGAGTAAG	801	54	[12]
VIM-2 R	CTACTCAACGACTGAGCG			
IMP F	GAGTGGCTTAATTCTCRATC	183	54	[13]
IMP R	CCAAACYACTASGTTATCT			
OXA-23 F	GTGGTTGCTTCTCTTTTTCT	737	54	[14]
OXA-23 R	ATTTCTGACCGCATTTCCAT			

820 isolates of *A. baumannii* (76.5%) and 252 isolates of *P. aeruginosa* (23.5%). In our study, there was a male predominance with a sex ratio M/F = 1.81. The mean age was 55.5 ± 15.2 years. The age distribution of patients seems to be heterogeneous with a peak around 60 years old for both species. The majority of our isolates were of hospital origin. Intensive care units were the main source of these two multiresistant isolates followed by surgical units (see Table 3). *A. baumannii* was isolated from brochopulmonary samples in 41% (n = 335/820), from urine in 17% (n = 139/820) and from blood cultures in 14.4% (n = 118/820). *P. aeruginosa* was isolated from bronchopulmonary samples in 36% (n = 90/252), from urine in 24% (n = 60/252) and from superficial pus in 14% (n = 36/252) see Table 3.

The study of the sensitivity of imipenem-resistant *A. baumannii* showed a resistance rate to ticarcillin of 100% (n = 820/820), piperacillin of 100% (n = 820/820), ticarcillin-clavulanic acid of 100% (n = 820/820), piperacillin-tazobactam 100% (n = 820/820), imipenem 100% (n = 820/820), Gentamicin 95% (n = 780/820), Amikacin 84% (n = 689/820), tobramycin 61% (n = 500/820), ciprofloxacin 98% (n = 803/820), and minocycline 74% (n = 607/820) see Table 4.

Imipenem-resistant *P. aeruginosa* isolates were resistant to ticarcillin in 80% (n = 202/252), piperacillin in 70% (n = 176/252), ticarcillin-clavulanic acid in 77% (n = 194/252), piperacillin-tazobactam in 65% (n = 164/252), ceftazidime in 69% (n = 173/252), imipenem in 100% (n = 252/252), gentamicin in 75% (n = 189/252), amikacin in 63% (n = 159/252) tobramycin in 70% (n = 176/252), ciprofloxacin in 70% (n = 176/252), meropenem in 94% (n = 237/252), aztreonam in 70% (n = 176/252) and colistin in 0% (0/252) see Table 4.

The carba test was positive in 16% (n = 17/108) isolates of *A. baumannii* against 62,5% (n = 10/16) isolates of *P. aeruginosa*.

The molecular study showed that out of 108 *A. baumannii* isolates analyzed, 102 (94.44%) carried the $bla_{\text{OXA-51}}$ gene and 100 isolates (92.6%) carried the $bla_{\text{OXA-23}}$ gene. These were the main carbapenemase enzymes in our *A. baumannii* collection. The coexistence of $bla_{\text{OXA-23}}$ and bla_{NDM} genes was detected in only 4 isolates (4%) and 3 isolates did not carry any of tested genes. Altogether 50% of *P. aeruginosa* carried $bla_{\text{VIM-2,}}$ see Fig. 1.

The $bla_{\text{OXA-23}}$ gene was furthermore isolated among patients of surgical resuscitation (38%), followed by medical resuscitation (21%). The $bla_{\text{VIM-2}}$ gene was furthermore isolated among patients from urology department (43%), followed by burned resuscitation (29%).

The $bla_{\text{OXA-23}}$ gene was isolated from broncho-respiratory samples in 55%, urinary samples in 17% and blood cultures in 12%. The $bla_{\text{VIM-2}}$ gene was furthermore isolated from urinary samples in 71% and superficial pus in 29%.

The resistance profile of *A. baumannii* isolates carrying the $bla_{\text{OXA-23}}$ gene is illustrated in Fig. 2 and that of *P. aeruginosa* carrying the $bla_{\text{VIM-2}}$ gene is illustrated in Fig. 3.

DISCUSSION

In recent decades, Gram-negative non-fermenting bacilli have been implicated in many infectious diseases. Although these are mainly associated with nosocomial infections, these bacteria have also been implicated in community acquired infections *P. aeruginosa* and *A. baumannii* represent today a particularly efficient adaptation model in terms of antibiotic resistance, its capacity to survive for a long time in hospital environment associated with the emergence of resistance potentiates its capacity of nosocomial propagation. In our study, there was a predominance of male sex in patients

Table 3. Sociodemographic and clinical data of patients related to imipenem-resistant *A. baumannii* and *P. aeruginosa* isolates

Epidemiological characteristics	imipenem-resistant *A. baumannii* (n = 820)		imipenem-resistant *P. aeruginosa* (n = 252)	
	number	%	number	%
Gender				
- Male	574	70%	169	67%
- Female	246	30%	83	33%
Age range				
- ≤17 years old	48	6%	30	12%
- 18-59 years old	298	36%	89	35%
- ≥ 60 years old	474	56%	133	53%
Patient's origin				
- Inpatient	648	79%	219	87%
- community	172	21%	33	13%
Hospitalization or admission department				
- medical services	121	14.8%	46	18%
- surgical services	123	15%	50	20%
- medical intensive care	211	25.7%	77	31%
- surgical intensive care	218	26.6%	44	18%
- emergency	122	14.9	29	11%
- external consultation	25	3%	6	2%
Nature of the sample				
- bronchopulmonary samples	335	41%	90	36%
- urine	139	17%	60	24%
- blood cultures	118	14.4%	13	5.2%
- profound pus	64	7.8%	25	10%
- superficial pus	50	6%	36	14%
- swabbing	55	6.7%	2	0.8
- materials	37	4.5%	16	6%
- puncture fluid	17	2.1%	2	0.8%
- biopsy	3	0.3%	5	2%
- coproculture	2	0.2%	3	1.2

Table 4. Resistance rates of imipenem-resistant *A. baumannii* and *P. aeruginosa* isolates

	imipenem-resistant *A. baumannii* (n = 820)		imipenem-resistant *P. aeruginosa* (n = 252)	
	Number of resistant isolates	%	Number of resistant isolates	%
Ticarcilin	820	100%	202	80%
Piperacillin	820	100%	176	70%
Ticarcillin/ clavulanic acid	820	100%	194	77%
Piperacillin/ tzaobactam	820	100%	164	65%
Cefepime	820	100%	166	66%
Ceftazidime	820	100%	173	69%
Imipenem	820	100%	252	100%
Gentamicin	780	95%	189	75%
Tobramycin	500	61%	176	70%
Amikacin	689	84%	159	63%
Netelmicin	648	79%	184	73%
Ciprofloxacin	804	98%	176	70%
Levofloxacin	803	98%	189	75%
Tetracyclin	738	90%	–	–
Minocyclin	607	74%	–	–
Trimethoprim-sulfamethoxazol	754	92%	–	–
Aztreonam	–	–	176	70%
Meropenem	–	–	237	94%
Colistin	0	0%	0	0%

infected with *A. baumannii* and *P. aeruginosa*. This predominance has been verified in other studies but the reason is not justified [15, 16].

The mean age of patients infected with *A. baumannii* and *P. aeruginosa* was 55.5 ± 15.2 years. However, our study showed that the most exposed age groups were the elderly (>60 years), which is in line with data reported by a study conducted in Brazil [17].

Fu Q et al. reported that an advanced age of furthermore 50 years can be considered as a risk factor predisposing to colonization or infection by pathogenic bacteria, due to the multiple defects and multi-visceral failures that are most often associated with advanced age and in particular hospitalization in intensive care which explains why these subjects are more likely to have nosocomial infections [18].

A. baumannii and *P. aeruginosa* are two well described nosocomial pathogens. *A. baumannii* is a bacterium that is more likely to contaminate the patient's environment, especially in intensive care units. It can persist for a long time and can be transmitted by nursing staff, hence hygiene measures and disinfection of the premises must be done regularly [3]. It was confirmed by our study, that *A. baumannii* isolates resistant to imipenem were present in the resuscitation services with a rate of 52.3%, followed by the medical and surgical services, the emergency room and then the diagnostic center. Our results were confirmed by other studies [19, 20]. This could be explained by the fact that many risk factors associated with Acinetobacter infection exist in intensive care units, such as the presence of potential environmental reservoirs for *A. baumannii*, colonized patients or patients with multiple wounds that are potential entry points, the misuse of broad-spectrum antibiotics, and the frequent contamination of the hands of health care personnel.

P. aeruginosa is a bacterium that is very common in health care facilities, it is present in water sources, and it is a potential contaminant of aqueous solutions as well as of mechanical ventilation equipment often found in intensive care units [21].

Our study showed that intensive care units represented 49% of our imipenem-resistant *P. aeruginosa* isolates. These results support the severity of these nosocomial germs which represent a real problem as much in our establishment as in other countries. In fact, the opportunistic nature of this bacterium and the presence of several favourable factors such as bed rest, the use of invasive devices as well as broad-spectrum antibiotic therapy mean that this bacterium is

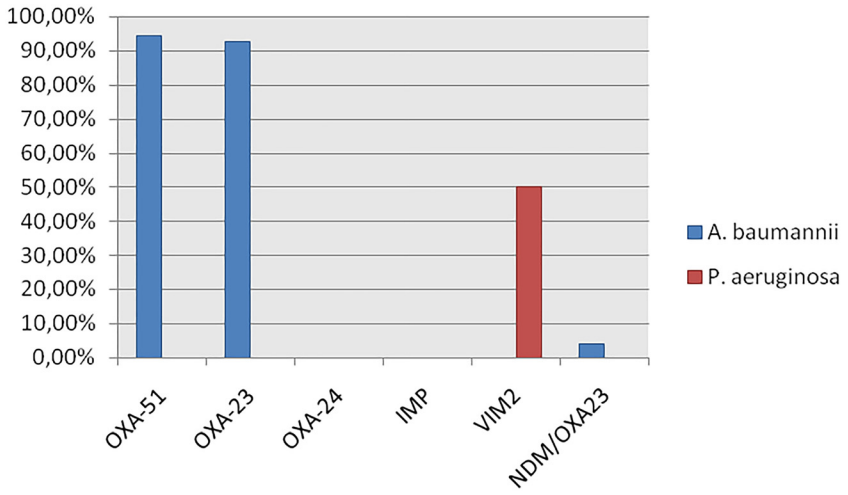


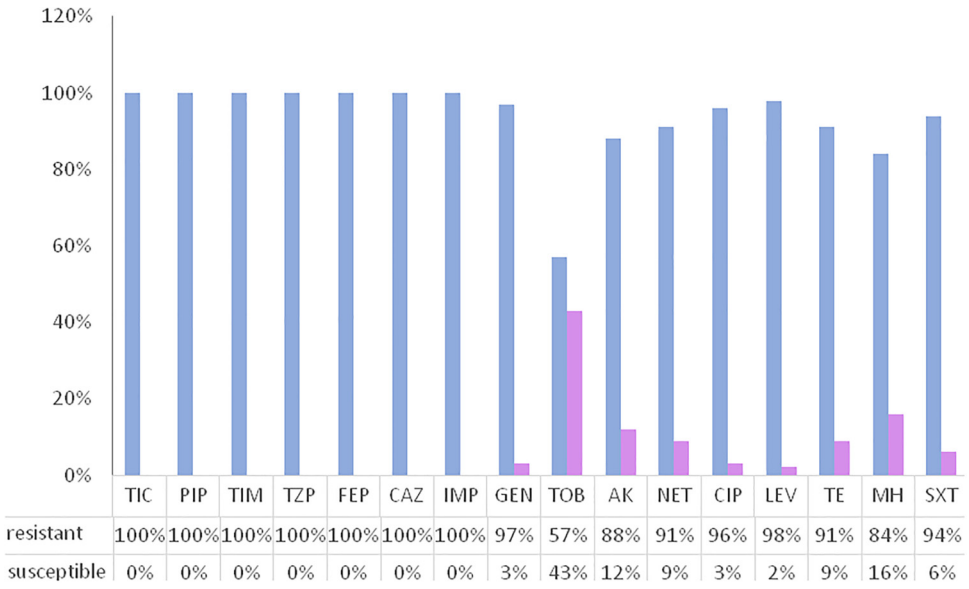
Fig. 1. Positivity rate of different carbapenemases genes.

responsible for infections much more in hospitals than in the community [22].

The results of our study showed a predominance of *A. baumannii* and *P. aeruginosa* strains in bronchopulmonary and urinary tract specimens, a finding that has been reported by many authors in their studies [3, 22]. This can be explained by the high fluid content of these anatomical sites, which constitute a favorable survival environment for these bacteria, or also by the presence of invasive devices such as urinary and endotracheal tubes, which are essential, especially in intensive care, at least on a temporary basis, and which can be easily colonized [23].

After bronchopulmonary and urinary samples, we find superficial pus samples with a percentage of 14% for *P. aeruginosa* isolates, as wounds are often moist, especially in burn victims, and this bacterium is very much involved in skin infections in this population, as the alteration of the skin, which represents a physical barrier, and the local reduction in the humoral immune response can lead to colonization of wounds by this bacterium. This rate shows the capacity of this bacterium to thrive in humid conditions, it can easily colonize water points and equipment close to the patient and can be transmitted by the personnel during care when hygiene measures are insufficient.

The β-lactams represent a family of antibiotics widely used in routine practice, but the high resistance of *A. baumannii* to this family compromises the efficacy of these antibiotics. The rate of resistance to ticarcillin, piperacillin, ticarcillin-clavulanic acid, piperacillin-tazobactam, ceftazidime, and cefepeme was 100%. These results were significantly higher



	TIC	PIP	TIM	TZP	FEP	CAZ	IMP	GEN	TOB	AK	NET	CIP	LEV	TE	MH	SXT
resistant	100%	100%	100%	100%	100%	100%	100%	97%	57%	88%	91%	96%	98%	91%	84%	94%
susceptible	0%	0%	0%	0%	0%	0%	0%	3%	43%	12%	9%	3%	2%	9%	16%	6%

Fig. 2. Resistance profile of *A. baumannii* isolates carrying the $bla_{\text{OXA-23}}$ gene.
TIC: ticarcillin; PIP: piperacillin; TIM: ticarcillin/clavulanic acid; TZP: piperacillin/tazobactam; IMP: imipenenme, FEP: cefepime; CAZ: ceftazidime; GEN: gentamicin; TOB: tobramycin; AK: amikacin; NET: netilmicin; CIP: ciprofloxacin; LEV: levofloxacin; TE: tetracycline; MH: minocycline; SXT: sulfamethoxazole+trimethoprim

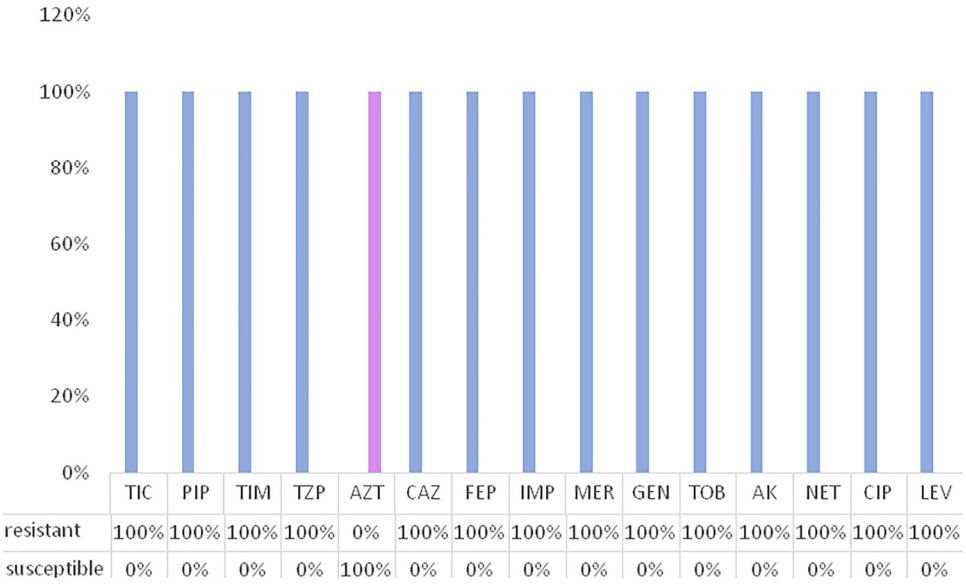


Fig. 3. Resistance profile of *P. aeruginosa* isolates carrying the $bla_{\text{VIM-2}}$ gene.
TIC: ticarcillin; PIP: piperacillin; TIM: ticarcillin/clavulanic acid; TZP: piperacillin/tazobactam; FEP: cefepime; CAZ: ceftazidime; IMP: imipenem; GEN: gentamicin; TOB: tobramycin; AK: amikacin; NET: netilmicin; CIP: ciprofloxacin; LEV: levofloxacin, MER: meropenem, AZT: aztreonam

than those found in our hospital in 2003 and 2017 [24, 25]. This could be explained by the uncontrolled and empirical prescription of these molecules.

For aminoglycosides, in our study, the rate of resistance to gentamicin and amikacin was 95% and 84%, respectively. These resistance rates are high compared to those reported in studies conducted at our institution [24, 25]. Tobramycin is an exception, the rate of resistance to this antibiotic has relatively decreased from 70.8% in 2003 to 43.03% in 2017 and 61% in our study [24, 25].

The resistance of *A. baumannii* to fluoroquinolones has always been reported in the literature, although resistance rate is stable in our institution between 1996 (68%) and 2003 (72%). It has increased up to 87.8% for ciprofloxacin as described in a study in 2017 [24], but this rate is close to ours in this study which was 98%. Fluoroquinolones are widely used both in hospitals and in outpatient settings, which favors the selection of strains with resistant genes with a risk of transmission of the latter. The new fluoroquinolones were effective, over the years however, with the inappropriate use their effectiveness is questioned. Our study reported a rate of 98% for levofloxacin resistance, and this rate is higher compared to that of Jaggi et al. in India who reported 87.6% [19].

For sulfamethoxazole/trimethoprim, the resistance rate increased from 83.1% in 2003 to 92% in our study. For tetracyclin the rate decreased slightly from 92.16% (2017) to 90% in our study [24, 25], this can be explained by the decrease in the use of these antibiotics at our facility. These high rates of resistance that affect the different families of antibiotics namely ß-lactams, aminoglycosides, tetracyclines are mainly due to the acquisition of mobile genetic carriers that are horizontally transferable and that lead to a spread of bacterial resistance [25].

Imipenem-resistant *P. aeruginosa* strains were found to be much less resistant than *A. baumannii*. The resistance rates found in our study were high and close to those in the literature [26, 27]. This increase in resistance rates can be explained by the fact that these molecules are often used in infections caused by the pyocyanic bacillus in an irrational and uncontrolled manner.

Among the imipenem resistant isolates we performed phenotypic testing on a number of isolates, 108 *A. baumannii* isolates and 16 *P. aeruginosa* isolates.

Resistance to colistin has not been observed for both species, which leads us to double the effort to preserve this molecule by avoiding the selection of resistant mutants and it remains the only available and effective molecule in the absence of innovation and commercialization of other molecules for the treatment of multidrug-resistant (MDR) bacteria infections.

Of the 108 *A. baumannii* strains tested with the CarbaNP test, 17 isolates were positive with a rate of 16%, for *P. aeruginosa* a much higher rate was noted with 10 positive isolates out of 16 tested (62.50%). However, this technique has a high sensitivity for class A and B carbapenemases and keeps a much lower sensitivity for class D carbapenemases which are very frequent in *A. baumannii*. These enzymes have a much lower activity than class A and B and do not completely hydrolyze imipenem and therefore their detection by the Carba NP test remains very limited [28].

At present, molecular methods are the only ones that allow precise characterization of the resistance enzymes produced by bacteria. In order to explain this resistance mechanism, we performed a genotypic study. Of the 108 *A. baumannii* isolates analyzed, 102 (94.44%) carried the $bla_{\text{OXA-51}}$ gene which is naturally present in this species and 100 (92.6%) carried $bla_{\text{OXA-23}}$. These are the main carbapenemase enzymes

produced by *A. baumannii* and the co-existence of the $bla_{\text{OXA-23}}$ and bla_{NDM} genes was detected in only 4 isolates (4%) and 3 strains carried no carbapenemase gene.

In the literature, the $bla_{\text{OXA-51}}$ gene, which is an intrinsic chromosomal gene in *A. baumannii*, is used for identification of the species and does not in any case reflect the acquisition of resistance to carbapenems [29]. However, 3 isolates in our series did not carry this gene and this can be explained by an error in identification on our part. Resistance to carbapenems by enzyme production was mainly due to OXA-23 with a percentage of 92.6%. The emergence of OXA-23 in Morocco is compatible with the global epidemiology of OXA-23 and with many reports from Mediterranean countries [30–33]. The $bla_{\text{OXA-23}}$ gene in *A. baumannii* has been described on several transposons, located on the chromosome or plasmids. All these mobile elements make the enzyme easily disseminable [25]. In our study, the $bla_{\text{OXA-23}}$ gene was isolated from broncho-respiratory samples in 55%, urinary samples in 17% and blood cultures in 12%.

However, rate of isolates producing NDM class B carbapenemases in *A. baumannii* remain lower in our study compared to previous studies in our country. One study was conducted in our institution and another in the city of Meknes, as well as other studies in Algeria and China reported higher rates than our study [25, 32, 34, 35].

Class B NDM carbapenemases have hydrolytic activity on all ß-lactams except aztreonam. The genes that code for these enzymes are located on mobile genetic elements that can be either transposons or plasmids and/or integrons, which give them a significant power of dissemination [25].

Molecular analysis of our *P. aeruginosa* isolates showed a rate of 50% for the $bla_{\text{VIM-2}}$ gene. Several other studies have shown the predominance of metallo-ß-lactamases with a lower rate than ours [36, 37]. Maroui I et al. reported the first isolation of bla_{VIM} genes in clinical isolates of *P. aeruginosa* in Morocco in 2016 [38].

The resistance profile of these isolates was 100% for all the families of antibiotics tested, except for aztreonam, which is explained by the fact that metallo-ß-lactamases do not hydrolyze this molecule because of their weak binding to it [39].

The emergence of VIM in *P. aeruginosa* can occur through the acquisition of different genes located on a class 1 integron that can be easily transferred between bacteria [40].

The information provided by the present study can show that it is an epidemic clone of *P. aeruginosa* producing metallo-ß-lactamase that is circulating in our hospital, hence the importance of regular monitoring of the local epidemiology, which allows measures to be taken to limit the spread of these bacteria and prevent nosocomial infections.

CONCLUSION

Gram-negative non-fermenting bacilli pose a real public health problem. Our study showed that resistance of *A. baumannii* and *P. aeruginosa* isolates to carbapenems is increasing in our region and this is becoming more and more alarming. The aim of our study was to review the local epidemiology of *A. baumannii* and *P. aeruginosa* infections as well as a molecular analysis. The resuscitation services were the first place in this study, which also showed that the most active antibiotics such as imipenem are very affected by these resistance mechanisms. Considering the impact of these bacteria on the vital prognosis of the patient and the socio-economic impact of the treatment of infections with multi-resistant bacteria, the alarm bell has been rung inviting the different actors of the health-care chain for a collective action and a mutual aid between clinician, microbiologist and pharmacist in order to improve the care of the patient and to better treat him in the future.

Authors' contributions statement: All authors have made substantial contributions. ME conceived, determined and designed the study. EB and TA acquired clinical and biological data. EB and ME analysed the data. EB, TA and AM presented and interpreted graphical data. EB and ME drafted, corrected and edited the manuscript. AM and TA performed the linguistic review and critically revised the manuscript. All authors read and approved the final version of the manuscript.

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Ethics approval and consent to participate: Requests for authorization to conduct the study and for collaboration in the research were addressed to the hospital director and the chief of the diagnostic center. As this was a retrospective study, in which the investigator had no contact with the patients, informed consent was not required. However, anonymity and confidentiality were respected for all study data.

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