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Susceptibility of multi-drug resistant isolates of *Pseudomonas aeruginosa* to ceftazidime/aviabactam and ceftolozane/tazobactam in Benha University Hospital in Egypt and detection of carbapenemase genes

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ABSTRACT

Background: Multidrug-resistant (MDR) *Pseudomonas aeruginosa* (*P. aeruginosa*) is a major public health threat. Ceftazidime/avibactam (CZA) and ceftolozane/tazobactam (C/T) are active against many MDR *P. aeruginosa*, and can improve the management of infected patients. However, resistance to the new antimicrobials has emerged in many countries. In this study we aimed to test the susceptibility of clinical isolates of MDR *P. aeruginosa* in our hospital to CZA and C/T, and to identify the role of carbapenemases in antimicrobial resistance. **Methods:** We studied 51 MDR *P. aeruginosa* isolates from 51 ICU patients admitted at Benha University Hospital in Egypt. Identification was done by VITEK-2 (BioMerieux). Testing the antimicrobial susceptibility was performed by disk diffusion method according to CLSI 2022. For all isolates, we used Carba Blue Test for phenotypic identification of carbapenemases, and multiplex PCR for detection of carbapenemase genes. **Results:** The isolates were 98% resistant to meropenem, while 23.5% were susceptible to each of C/T and CZA. Carbapenemase production was common, mostly by metallo- beta- lactamases (MBL). The most common carbapenemase genes were NDM, VIM and SIM. Carba Blue test identified carbapenemases in 52.9%, while multiplex PCR identified carbapenemase genes in 91.1%. **Conclusion:** Compared to conventional antibiotics, both CZA and C/T have shown moderate efficacy against our MDR *P. aeruginosa* isolates, making them good choices for the treatment of infection by some MDR *P. aeruginosa*. PCR is more sensitive than Carba Blue in detection of carbapenemase production.

Introduction

Pseudomonas aeruginosa has been the causative agent of significant clinical infections, including sepsis, central line-associated bloodstream infections (CLABSI), ventilator-associated pneumonia (VAP), bacteremia, surgical site infections (SSI), infected burns (inf b), skin and skin structure infections, hospital- acquired pneumonia (HAP), and catheter- associated urinary tract infections (CAUTI). It was estimated that at least 7.5% of healthcare associated infections were caused by *P. aeruginosa*, including 16.6% of VAP, 11.3% of CAUTI, 5.5% of SSI, and 3.8% of CLABSI leading to high rates of morbidity and

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mortality [2, 3]. *Pseudomonas aeruginosa* has developed a variety of intrinsic mechanisms to respond to harmful environmental threats, including antibiotics including biofilm formation and intrinsic resistance to some antimicrobials. The acquisition of antibiotic resistance by *P. aeruginosa* is quite diverse [4].

The mechanisms of resistance in *P. aeruginosa* include the loss of an outer membrane porins (OprD), an inducible chromosomal beta-lactamase (AmpC), over-expression of multiple bacterial efflux pumps, and production of beta lactamases, usually metallo-enzymes including VIM-, IMP- and NDM, and ESBLs, including PER-1, VEB, GES, TEM, SHV and CTX-M. These resistance mechanisms led to evolution of *P. aeruginosa* strains that are extensively drug resistant (XDR) and multidrug resistant (MDR) [4, 5]. In the Arab World, the main mechanism of resistance is production of beta-lactamases, in contrast to the situation in Europe and USA, where the other resistance mechanisms dominate [5].

In Egypt, *P. aeruginosa* infections account for more than 15.7% of ICU patients in Egypt including 57% MDR *P. aeruginosa* [6]. Resistance to carbapenems is the commonest phenotype of resistance in *P. aeruginosa*, accounting for 15–70% [7]. Because beta-lactamase production frequently results in the resistance to all beta-lactam antimicrobials, carbapenemase producing isolates frequently exhibit MDR phenotypes. Infection by MDR isolates restricts therapeutic options and is associated with higher patient risk of morbidity and mortality [3,5].

In 2020, avibactam, which is a non- β-lactamase β-lactamase inhibitor, was introduced in order to restore antimicrobial activity against isolates of Gram-negative bacilli harboring Ambler class A and D beta-lactamases, including extended spectrum β -lactamases and serine- carbapenemases [8]. Adults with complicated urinary tract infections, such as pyelonephritis, complicated intra-abdominal infections, hospital-acquired pneumonia, and other infections caused by *Enterobacterales* and *P. aeruginosa*, could be treated with the combination of ceftazidime and avibactam [8].

In 2018, ceftolozane, a cephalosporin with improved anti- *P. aeruginosa* efficacy, was coupled with tazobactam, a well-known beta-lactamase inhibitor. Ceftolozane-tazobactam has been proven to be both safe and effective for treating severe urinary tract infections and complicated intraabdominal infections brought on by *Enterobacterales* and *P. aeruginosa* [9].

The aim of this study was to detect susceptibility of MDR *P. aeruginosa* clinical isolates in our hospital to the antimicrobials, CZA and C/T, and to identify carbapenemase production and carbapenemase genes among the MDR *P. aeruginosa isolates*.

Patients and methods

Study design

This was a cross-sectional study done between March 2021 and February 2022, on 51 consecutive, non-duplicate clinical isolates of MDR *P. aeruginosa* isolates from intensive care unit (ICU) patients hospitalized at Benha University hospital. All laboratory procedures were performed in the microbiology laboratory of Benha University Hospital except for detection of the carbapenemase genes, which was done in the Molecular and Research Microbiology Laboratory in the Clinical Pathology Department, Faculty of Medicine, Cairo University, Egypt.

Ethical approval

The study was approved by the Institutional Review Board of the Faculty of Medicine of Benha University. Obtaining consent was not required as the isolates were collected as part of routine clinical care, and no diagnostic or treatment decisions were affected by the outcomes of this study. All specimens and relevant data were de-identified to keep patients' confidentiality.

Specimen collection

Clinical specimens from ICU patients with suspected infections were submitted to the microbiology laboratory, according to our hospital policy, for identification of pathogens by culture on blood agar, chocolate agar, MacConkey agar media and in-vitro susceptibility testing by standard procedures [10]. All media were purchased as dehydrated powder media from Thermo Scientific™ Oxoid™ and prepared in the hospital laboratory, according to the manufacturer's instructions.

Identification of *P. aeruginosa*

We identified *P. aeruginosa* by the characteristic colony morphology and Gram stain. Identification was confirmed by testing biochemical and enzymatic reactions by VITEK-2 system (Biomerieux) [11].

Antimicrobial susceptibility testing

The antibiotic susceptibility test was done using disk diffusion method according to the recommendations of the Clinical Laboratory Standards Institute (CSLI 2022), including ceftazidime-avibactam 30/20ug, ceftolozane tazobactam 30/10ug [12].

Susceptibility to imipenem 10 µg (IPM), meropenem 10 µg (MEM), aztreonam 30 µg (ATM), ceftazidime 30 µg (CAZ), cefepime 30 µg (FEP), piperacillin/tazobactam 100/10 µg (TZP), ciprofloxacin 5 µg (CIP), amikacin 30 µg (AK) and gentamicin 10 µg (CN) disks was determined through the disk-diffusion method following CLSI 2022 recommendations; using Mueller Hinton agar (Thermo Scientific™ Oxoid™), and incubation for 16-18 hours at 35+2C in ambient air [12].

Quality control (QC) tests were performed using the reference strain *P. aeruginosa* ATCC 27853, and all QC were within the CLSI acceptable range. The phenotypes "susceptible", "intermediate" and "resistant" were defined for each antibiotic based on their respective zone diameter breakpoints established by CLSI 2022) [12].

Detection of carbapenemase production

To explore the role of carbapenemase production in carbapenem resistance, phenotypic tests for carbapenemase activity (Carba Blue) and molecular tests (polymerase chain reaction, PCR) for detection of genes were performed.

Isolates showing resistance to imipenem (IMI), meropenem (MEM), or both were selected for testing carbapenemase activity and identifying the carbapenemase genes.

We performed an in-house Carba Blue test as previously described [13]. Briefly, a 5-µl loop of the bacterial culture isolated in Mueller-Hinton agar was suspended in two different 0.04% bromothymol blue solutions containing (test) or not containing (control) 3 mg/ml imipenem (imipenem 500; Merck Sharp & Dohme, France) plus 0.1 mmol/liter ZnSO4, and color changes were registered after an incubation period at 37°C for 2 hours.

For detection of carbapenemase genes, DNA was extracted from *P. aeruginosa* colonies by the simple boiling method and the concentration of extracted DNA was determined at 260 nm, using Nanodrop instrument (ThermoFisher Scientific, Waltham, MA, USA). We used 3 sets of multiplex PCR testing for identification of carbapenemase genes. Primers were used to amplify the following 11 genes: blaIMP, blaVIM, blaNDM, blaSPM, blaAIM, blaDIM, blaGIM, blaSIM,blaKPC, blaBIC, and blaOXA-48. Three different multiplex reaction mixtures were defined and evaluated for the detection of all these 11 genes, with reaction no. 1 for detection of blaIMP, blaVIM, and blaSPM, reaction no. 2 for detection of blaNDM, blaKPC, and blaBIC, and reaction no. 3 for detection of blaAIM, blaGIM, blaSIM, and blaDIM [14]. PCR mixture was prepared in a final volume of 25 µL comprising 10× PCR buffer, 1.5 mmol of MgCl2, 0.2 mmol of each dNTPs, 5 U/µL of Taq DNA polymerase, 1 µmol of each primer, 8 µL of distilled water, and 8 ng of template DNA. The amplification was carried out in a thermocycler (Eppendorf, Germany) with the following cycling conditions: initiation denaturation at 95°C for 10 minutes, and 36 cycles of denaturation at 95°C for 30 seconds, annealing at 52°C for 40 seconds, extension at 72°C for 50 seconds, and a final extension at 72°C for 5 minutes. DNA fragments were analyzed by electrophoresis on a 2% agarose gel containing 0.5 µg/mL ethidium bromide. The bands were visualized under UV light using a gel documentation system (Protein Simple, USA). The primers are listed in **table (5)** in supplementary material as previously reported [14].

Statistical analysis

The collected data was statistically analyzed using SPSS program (Statistical Package for Social Science) version 26. Data was tested for normal distribution using the Shapiro Walk test. Qualitative data was represented as frequencies and relative percentages. Quantitative data was expressed as mean and standard deviation. Level of p-value < 0.05 indicates significant while, $p \geq 0.05$ indicates non-significant difference [15].

Results

Demographic criteria

A total number of 51 clinical isolates of MDR *P. aeruginosa* from ICU patients were identified and enrolled in the study. The mean age of patients was 58.2± 18.2 years ranging from 22 to 90 years with a median of 63 years. Patients were 47.1% males and 52.9% females.

Clinical diagnosis was catheter-associated urinary tract infections (CAUTI) in 27.5%, central line associated bloodstream infections in 13.7%, Ventilator associated pneumonia in 13.7%, infected burn in 13.7%, surgical and site infection in 1.4%. The sources of specimen were blood (13.7%), endotracheal tube (13.7%), pus (45.1%) and urine (27.5%).

Antimicrobial susceptibility

Resistance to meropenem (MEM) was identified in 98% resistant, 2% were intermediate, and 0% sensitive. Resistance to imipenem (IPM) was 96.1% 3.9% were intermediate, and 0% susceptible. High rates of resistance were detected to other antimicrobials. The rates of resistance were 82.4% to amikacin (AK), 86.3% to piperacillin/ tazobactam (TZP), 90.2% to levofloxacin (LEV), and 84.3% to ceftazidime (CAZ). For CZA, 23.5% of isolates showed susceptibility and 76.5% were resistant. For C/ T, 23.5% of isolates showed susceptibility, while 66.7% were resistant and 9.8% showed intermediate susceptibility **(Figure 1).**

Of 51 isolates, 42 isolates (82.4%) were defined as difficult-to-treat resistance (DTR), as they were non-susceptible to all beta-lactams and quinolones; 5 isolates (9.8%) were extensively drug-resistant (XDR), as the isolates were non-susceptible to five antibiotic families or more, and not considered DTR; and 4 isolates (7.8%) were MDR, non-DTR and non-XDR isolates presenting a non-susceptible phenotype to three or four antibiotic families.

Testing for carbapenemase activity

The Blue Carba phenotypic test was positive among 27 (52.9%) There was no statistically significant association between CZA or C/T resistance and positive Blue Carba *p* value 0.371 and 0.423 respectively **(Table 1).**

Carbapenemase genes

Carbapenemase genes were detected in 48 (91.1%) of the isolates, indicating that carbapenemase production is a common resistance mechanism. Three isolates were negative for all tested carbapenemase genes, suggesting different resistance mechanisms. The predominant genes were NDM, VIM and SIM **(Table 2).** There was a statistically significant association between resistance to C/ T and NDM, GIM and SIM, and no statistically significant association between resistance to C/ T and OXA, KPC, VIM, IMP, SPM, AIM and DIM **(Table 3).** There was statistically significant association between resistance to CZA and NDM and SIM, and no statistically significant association between resistance to C/ T and OXA, KPC, VIM, IMP, SPM, AIM, GIM and DIM **(Table 4).**

Carba Blue test was negative in 18 of our isolates that harbored carbapenemase genes. No false positive results were observed. The distribution of the resistance genes in Carba Blue negative isolates is shown in **figure (2).**

Table 1. Association between Blue carba and CZA and C/T results.

Antibiotic		Blue Carba		*P* value
		Negative	Positive	
CZA	**Resistant**	17 (70.8)	22 (81.5)	0.371[a]
	Sensitive	7 (29.2)	5 (18.5)	
C/T	**Intermediate**	1 (4.2)	4 (14.8)	0.423[b]
	Resistant	16 (66.7)	18 (66.7)	
	Sensitive	7 (29.2)	5 (18.5)	

a; Chi square test, b; Fisher Exact test, *p* value is significant at <0.05.

Table 2. Distribution of resistance genes among the 51 isolates of *Pseudomonas aeruginosa*.

Resistance genes	Positive		Negative	
	n	%	N	%
NDM	34	66.7	17	33.3
VIM	18	35.3	33	64.7
SIM	18	35.3	33	64.7
OXA	12	23.5	39	76.5
GIM	5	9.8	46	90.2
KPC	4	7.8	47	92.2
AIM	3	5.9	48	94.1
SPM	2	3.9	49	96.1
IMP	2	3.9	49	96.1
DIM	0	0	51	100

Table 3. Association between resistance to C/T and carbapenemase genes.

C/T		Sensitive n= 12		Resistant n= 34		Intermediate n= 5		*P* value
		n	%	N	%	n	%	
NDM	**Negative**	8	66.7	8	23.5	1	20	0.020*
	Positive	4	33.3	26	76.5	4	80	
OXA	**Negative**	9	75	26	76.5	4	80	>0.999
	Positive	3	25	8	23.5	1	20	
KPC	**Negative**	10	83.3	32	94.1	5	100	0.578
	Positive	2	16.7	2	5.9	0	0	
VIM	**Negative**	7	58.3	23	67.6	3	60	0.903
	Positive	5	41.7	11	32.4	2	40	
IMP	**Negative**	10	83.3	34	100	5	100	0.060
	Positive	2	16.7	0	0	0	0	
SPM	**Negative**	10	83.3	34	100	5	100	0.060
	Positive	2	16.7	0	0	0	0	
GIM	**Negative**	12	100	31	91.2	3	60	0.049*
	Positive	0	0	3	8.8	2	40	
SIM	**Negative**	12	100	18	52.9	3	60	0.011*
	Positive	0	0	16	47.1	2	40	
AIM	**Negative**	12	100	31	91.2	5	100	0.677
	Positive	0	0	3	8.8	0	0	
DIM	**Negative**	12	100	34	100	5	100	-
	Positive	0	0	0	0	0	0	

Fisher Exact test; *p is significant at <0.05

Table 4. Association between resistance CZA and carbapenemase genes.

CZA		Resistant n= 39		Sensitive n= 12		*P* value
		N	%	n	%	
NDM	**Negative**	9	23.1	8	66.7	0.008*
	Positive	30	76.9	4	33.3	
OXA	**Negative**	30	76.9	9	75	0.584
	Positive	9	23.1	3	25	
KPC	**Negative**	37	94.9	10	83.3	0.232
	Positive	2	5.1	2	16.7	
VIM	**Negative**	26	66.7	7	58.3	0.732
	Positive	13	33.3	5	41.7	
IMP	**Negative**	39	100	10	83.3	0.052
	Positive	0	0	2	16.7	
SPM	**Negative**	39	100	10	83.3	0.052
	Positive	0	0	2	16.7	
GIM	**Negative**	34	87.2	12	100	0.323
	Positive	5	12.8	0	0	
SIM	**Negative**	21	53.8	12	100	0.002*
	Positive	18	46.2	0	0	
AIM	**Negative**	36	92.3	12	100	0.573
	Positive	3	7.7	0	0	
DIM	**Negative**	39	100	12	100	-
	Positive	0	0	0	0	

Fisher Exact test; **p* is significant at <0.05

Figure 1. Susceptibility and resistance percentage of different antimicrobials against 51 *Pseudomonas* isolates. Abbreviations: MEM: meropenem, IPM: imipenem, AK: amikacin, LEV: levofloxacin, CAZ: ceftazidime, CZA: ceftazidime/ avibactam, C/T: ceftolozane/ Tazobactam.

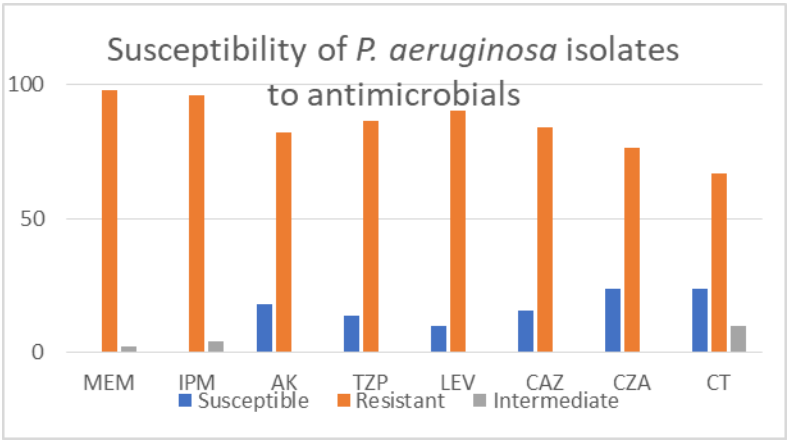
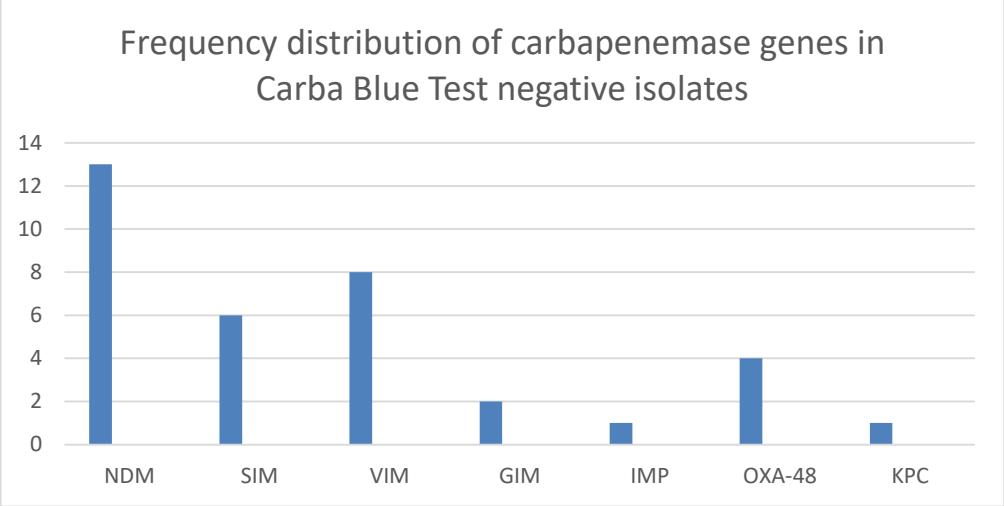


Figure 2. Frequency distribution of carbapenemase genes in Carba Blue Test negative isolates.



Discussion

Fifty-one consecutive, non-duplicate clinical isolates of MDR *P. aeruginosa* from ICU patients were tested for antimicrobial susceptibility by disk diffusion methods according to CSLI, 2022 [12].

The source of the isolates were adult patients (mean age 58.2± 18.2 years). They included 47.1% males. Clinical diagnoses were CAUTI (27.5%), CLABSI (13.7%), VAP (13.7%), infected burn (13.7%), and SSI (1.4%). This is in agreement with previous reports that *P. aeruginosa* is a cause of a substantial proportion of nosocomial infections [1- 3, 6,7].

The isolates had high rates of resistance to many antimicrobials, including 98% resistance to Meropenem, 96.1% resistance to Imipenem, 82.4% resistance to amikacin, 86.3% resistance to piperacillin/ tazobactam and 90.2% resistance to levofloxacin. Forty-two isolates (82.4%) were classified as DTR, as they were non-susceptible to all beta-lactams and quinolones; 5 isolates (9.8%) were XDR. Similar results were reported from Iran [16] with MDR and XDR *P. aeruginosa* isolates reported as 50% and 40% respectively. This is in keeping with the high rates of antimicrobial resistance in P. aeruginosa previously reported from the Arab World [5], with resistance to third generation cephalosporins, fluoroquinolones and carbapenems exceeding 50% [5,17].

Carbapenem resistance in *P. aeruginosa* can be mediated by extrinsic mechanisms, such as acquired carbapenemases, including the Ambler class B metallo-β-lactamases (MBL) and *Klebsiella pneumoniae* carbapenemases (KPC). The intrinsic mechanisms include overexpression of chromosomal cephalosporinases and hyperexpression of MexA-B and MexX-Y efflux pumps and OprD outer membrane protein mutations, which frequently occur simultaneously in *P. aeruginosa* [4, 5].

Both ceftazidime-avibactam (CZA) and ceftolozane–tazobactam (C/T) have shown activity against most carbapenem-resistant *P. aeruginosa* infections in Western Europe, where 23.1% of P. aeruginosa isolates were MDR. Of these, 72.0% were ceftolozane/tazobactam-susceptible, which is similar to that for ceftazidime/avibactam (73.6%). Acquired β-lactamases were not identified in 80.0% of molecularly characterized MDR *P. aeruginosa* isolates.[18]. Similarly, in the study of **O'Neall et al.** the susceptibility rates of carbapenem-resistant MDR *P. aeruginosa* to both CZA and C/T were comparable, at 66.4% and 67.6% respectively [19]. However, higher susceptibility of *P. aeruginosa* to C/T was reported by **Humphries et al.** with 61.8% susceptibility to CZA compared to 72.5% susceptibility to C/T, and 36.4% of CZA -resistant isolates were susceptible to C/T. The authors explained that C/T has several advantages over CZA including greater affinity for penicillin-binding proteins produced by *P. aeruginosa*, better membrane permeability, greater stability against Pseudomonas AmpC β-lactamases, up-regulated efflux pumps and loss of porins [20].

Testing our isolates with CZA and C/T showed 23.5% susceptibility to each of the 2 antimicrobials, while resistance to CZA was in 76.5%. Resistance to C/T was in 66.7%, with intermediate susceptibility in 9.8%. Both CZA and C/T showed more activity against our isolates and lower rates of resistance compared to other tested antimicrobials. The reduced activity of CZA and C/T against our isolates is consistent with high rates of carbapenemase production by *P. aeruginosa* in some countries, especially MBL production. This is in contrast to the situation in Europe and the USA, where most antimicrobial resistance in *P. aeruginosa* is attributable to AmpC and over-expression of efflux pumps [4, 5]. Yet the rates of resistance in our institution are higher than previous reports, reflecting the selection of MDR isolates for the study, the severity of underlying diseases of the ICU patients, and the overuse and misuse of antimicrobials in the hospital and community that led to a high prevalence of MDR bacteria among the population in Egypt [21]. This is coupled with inadequate compliance with infection control and antimicrobial stewardship in hospitals [6]. In agreement, there is high prevalence of MDR *P. aeruginosa* in the majority of the countries in the Middle East and North Africa region with similarities between neighboring countries, which might echo comparable population and antibiotic-prescribing attitudes and practices. Isolates from critical care units are significantly resistant particularly from certain countries such as Saudi Arabia, Egypt, Libya, Syria, and Lebanon with high-level resistance to cephalosporins, carbapenems, and aminoglycosides [22].

Detection of carbapenemases

Early detection of carbapenemase production can support optimal antimicrobial treatment of MDR P. aeruginosa, as carbapenemase non- producers are more likely to be susceptible to CZA and C/T [19,20]. Carbapenemase production can be detected in the laboratory by many phenotypic and genotypic methods. Among the phenotypic methods the Carba Blue test has been evaluated and found to be both rapid, accurate and cost-effective [23]. However, the test may give false negative result with week or low-level carbapenemase production, especially with metallo-beta lactamases (MBL) [24]. So, we tested 51 MDR *P. aeruginosa* isolates both by Carba Blue and multiplex PCR.

Novais et al. [25], evaluated the performance of Carba Blue rapid test against 75 carbapenemase producing isolates from different Enterobacteriaceae (*E. coli*, *K. pneumoniae*, *E. cloacae*), *Pseudomonas* spp. (*P. aeruginosa*, *P. pseudoalcaligenes*) and *Acinetobacter* spp. (*A. baumannii*, *A. pittii*, *A. haemolyticus*) species, and 30 non-carbapenemase producers (including extended-spectrum β-lactamase- and/or AmpC-producing isolates) with or without alterations in outer membrane permeability. The Rapid Carb Blue kit detected 93.3% of carbapenemase producers, false-negative results being obtained for 2 *P. aeruginosa* isolates producing VIM-2 or GES-6. No false positive results were detected.

In our study, the in-house Blue Carba test was positive among only 27 (52.9%) of isolates, and was negative in 18 of our isolates that harbored carbapenemase genes (dominated by NDM, VIM, SIM and OXA-48), probably due to low- level carbapenemase production. We didn't encounter false positive results. The sensitivity of Blue Carba test on our isolates was much inferior to previous results [25]. This difference reflects the variation in the tested isolates, resistance mechanisms and inter-operator variability, and pointing to the importance of modification and standardization of this test [25]. Thus a negative test result requires confirmation by another phenotypic or genotypic method for detection of carbapenemases [26].

We used 3 sets of multiplex PCR testing for genotypic identification of carbapenemases genes. Our results identified NDM, OXA-48, KPC, VIM, IMP and SPM in 66.7 %, 23.5 %, 7.8 %, 35.3%, 3.9 % 3.9 % of the isolates, respectively, which elucidates that MBL are the most frequent genes identified in our study. There was a statistically significant association between resistance to C/ T and the NDM, GIM and SIM genes, and between resistance to CZA and the NDM and SIM genes. There was no statistically significant association with other carbapenemase genes. These associations confirmed resistance to both CZA and C/T among MBL- producing bacteria.

This is in keeping with reports from Iran, where blaNDM (32%) dominated [16]. In Hungary the isolates expressing NDM showed the strongest possible resistance to all β -lactam antibiotics that were tested. The rapid spread of VIM and NDM carbapenemases among many species of Gram-negative bacteria could soon end most of the

currently available and newly developed antimicrobial therapeutic options [27]. The predominance of MBL was also reported **by Bitar et al.** [28]. **O'Neall et al.** reported that VIM was the most predominant (20.8 %) in their isolates while NDM was detected only in 2.8 % [23].

Conclusion

Our isolates showed high rates of antimicrobial resistance among the MDR *P. aeruginosa* isolates from ICU patients. Susceptibility to each of CZA and C/T was 23.5%, but higher than the susceptibility to other antimicrobials. Carbapenemase genes were frequently detected (91.1%), with predominance of MBL genes. Blue Carba test detected carbapenemases in only 52.9 % of the isolates. A combined local lab algorithm for rapid detection of carbapenemase- producing organisms can combine Carba Blue test with other phenotypic and molecular tests to improve sensitivity.

Limitations

Include the small sample size and being from one center.

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Competing nterests

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