Antimicrobial resistance pattern and molecular genetic distribution of metallo-β-lactamases producing *Pseudomonas aeruginosa* isolated from hospitals in Minia, Egypt

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**Background:** *Pseudomonas aeruginosa* (*P. aeruginosa*) represents a great threat to public health worldwide, due to its high ability to acquire resistance to different antibiotic classes. Carbapenems are effective against multidrug resistant (MDR) *P. aeruginosa*, but their widespread use has resulted in the emergence of carbapenem-resistant strains, which is considered a major global concern. This study aimed to determine the prevalence of carbapenem resistance among *P. aeruginosa* strains isolated from different sites of infection.

**Methods:** Between October 2016 and February 2018, a total of 530 clinical specimens were collected from patients suffering from different infections, then processed and cultured. Isolates were tested for extended spectrum β-lactamase (ESBL) and metallo-β-lactamase (MBL) production using double-disk synergy test, modified Hodge test, and disc potentiation test. PCR was used for the detection of selected OXA carbapenemases encoding genes.

**Results:** Of 530 samples, 150 (28.3%) *P. aeruginosa* isolates were obtained. MDR strains were found in 66.6% (100 of 150) of isolates. Of 100 MDR *P. aeruginosa* isolates, 54 (54%) were ESBL producers and 21 (21%) carbapenem resistant *P. aeruginosa*. MBL production was found in 52.3% (eleven) carbapenem-resistant isolates. CTX-M15 was found among 55.5% of ESBL-producing *P. aeruginosa*. Carbapenemase genes detected were *blaIMP* (42.8%, nine of 21), *blaVIM* (52.3%, eleven of 21), *blaGIM* (52.3%, eleven of 21), and *blaSPM* (38%, 8/21). In addition, isolates that were positive for the tested genes showed high resistance to other antimicrobials, such as colistin sulfate and tigecycline.

**Conclusion:** Our study indicates that *P. aeruginosa* harboring ESBL and MBL with limited sensitivity to antibiotics are common among the isolated strains, which indicates the great problem facing the treatment of serious infectious diseases. As such, there is a need to study the resistance patterns of isolates and carry out screening for the presence of ESBL and MBL enzymes, in order to choose the proper antibiotic.

**Keywords:** MDR; *P. aeruginosa*, ESBL, MBL, antimicrobial resistance

**Introduction**

*Pseudomonas aeruginosa* is an opportunistic pathogen that can cause outbreaks of hospital-acquired and life-threatening infections, especially among immunocompromised and critically ill patients.1 *P. aeruginosa* can cause respiratory tract, burn, wound infections and otitis media.2 *P. aeruginosa* infections are commonly associated with high mortality, attributed to its intrinsic resistance to many classes of antimicrobial agents and ability to acquire resistance by mutation and horizontal
transfer of resistance determinants. The rapid emergence of penicillin and cephalosporin resistance among *P. aeruginosa* strains has become a serious clinical problem worldwide. Carbapenems (imipenem and meropenem), potent antipseudomonal drugs, have been used as the last resort for the treatment of infections associated with multidrug resistant (MDR) *P. aeruginosa* isolates. Resistance to carbapenems has developed through decreased permeability, overexpression of the efflux-pump system, alterations in penicillin-binding protein and carbapenem-hydrolyzing enzymes (carbapenemases).

Carbapenemases represent three classes of β-lactamase (BL). Ambler class A and D (serine carbapenemases) and class B (zinc-dependent). These enzymes require zinc for their catalytic activity and are inhibited by metal chelators, such as EDTA and thiol-based compounds, and are called metallo-BLs (MBLs). MBL enzymes are able to hydrolyze all β-lactam antibiotics, with the exception of monobactams. The genes encoding these enzymes have found to be carried on highly mobile elements, which is the main cause of their dissemination in the hospital environment. MBLs are mainly plasmid-mediated and in some cases chromosomally mediated. The most common MBLs enzymes belong to the Verona integron-encoded MBL (VIM), imipenemase (IMP), São Paulo MBL (SPM), German imipenemase MBL (GIM), Seoul imipenemase MBL, and New Delhi MBL families.

Infections caused by MBL-producing organisms are associated with high morbidity and mortality rate, especially in hospitalized and immunosuppressed patients. Recently, many studies reported the prevalence of *P. aeruginosa* strains harboring both extended-spectrum BL (ESBL) and MBL genes, which is considered a great challenge for antimicrobial therapy. In addition, it is difficult to detect ESBLs phenotypically. As such, molecular techniques are required to analyze the coexistence of carbapenemases and ESBLs in the same strain. The aim of this study was to study the prevalence and DR profile of carbapenem-resistant *P. aeruginosa* (CRPA) isolates obtained from hospitalized patients with various infections.

**Methods**

**Bacterial isolates**

A total of 150 (28.3%) *P. aeruginosa* isolates were isolated from 530 samples collected from hospitalized patients with various infections as part of routine hospital-laboratory procedures. Samples were processed and cultured on blood agar at 37°C and 42°C for 24 hours. One colony was picked and subcultured on MacConkey agar plates and cetrimide agar. Isolated colonies were further identified according to colony morphology, lactose fermentation, and biochemical characteristics (oxidase, triple sugar iron, urease tests, sulphide–indole–motility). Colonies were able to grow on cetrimide agar, show positive reactions on catalase and oxidase tests, grow at 42°C (used to distinguish *P. aeruginosa* from other lactose nonfermenting Gram-negative rods), and show negative results in triple-sugar iron and glucose-fermentation tests.

*P. aeruginosa* colonies were purified by streaking, and pure colonies were stored at 4°C.

**Antimicrobial-susceptibility testing**

Antimicrobial susceptibility was determined by Kurby–Bauer disk diffusion test. Results were assessed on the basis of Clinical and Laboratory Standards Institute criteria. The following antimicrobial disks (Oxoid, Basinstoke, UK) were used: azlocillin (75 µg), ciprofloxacin (5 µg), ampicillin–sulbactam (20 µg), levofloxacin (5 µg), cefepime (30 µg), meropenem (10 µg), aztreonam (30 µg), imipenem (10 µg), polymyxin B (300 µg), colistin sulfate (10 µg), tigecycline (15 µg), tobramycin (10 µg), ceftazidime (30 µg), amoxicillin–clavulanic (20/10 µg), carbenicillin (100 µg), amikacin (30 µg), gentamicin (10 µg), piperacillin (100 µg), and cefoperazone (75 µg).

**Phenotypic detection of ESBL production**

Detection of ESBL production by *P. aeruginosa* strains was performed by double-disk synergy test (DDST). Disks of ceftazidime, cefotaxime, aztreonam, and cefepime (30 µg each) were placed at a distance of 30 or 20 mm (center to center) from an amoxicillin 20 µg–clavulanic acid 10 µg disk. Increase in zones of inhibition toward amoxicillin–clavulanic acid antibiotic disks is indicative of the presence of ESBL.

**Phenotypic detection of MBL production**

Imipenem–EDTA combined disk synergy testing was used for identification of MBL-producing isolates according to Lee et al. A solution of 0.5 M EDTA (pH 8) was prepared by dissolving 18.61 g of EDTA in 100 mL distilled water and adjusting its pH to 8 using NaOH, then, autoclaving. The tested organisms were cultured on the surface of Müller–Hinton agar plates. Two 10 µg imipenem disks or two 10 µg meropenem disks were placed on the surface of agar plates and 5 µL EDTA solution added.
to one imipenem and one meropenem disks. Zones of inhibitions around discs with EDTA were examined after 16–18 hours’ incubation at 35°C and compared to those without EDTA. An increase in zone diameter of at least 7 mm around the imipenem–EDTA disc and meropenem–EDTA disks were considered positive results.

Amplification of ESBL-CTX-M15 and MBL genes
Boiling was used to prepare DNA templates of genes. Specific primers — cefotaximase (bla<sub>CTX-M15</sub>), bla<sub>VIM</sub>, bla<sub>IMP</sub>, bla<sub>GIM</sub>, and bla<sub>SPM</sub> (Table 1) — were used for PCR amplification of the genes. PCR amplification was done using 25 µL reaction mixture containing 0.2 µL Taq polymerase 5 U/µL 1 pmol of each forward and reverse primer, 2.5 µL dNTP mix (2 Mm), 3 µL DNA template, and 14.8 µL DNase-free and RNase-free water. PCR reactions were performed using a Mastercycler personal 5332 (Eppendorf, Hamburg, Germany). Amplified products were analyzed by electrophoresis in 2% agarose gel at 80 V for 45 minutes in Tris–Borate–EDTA buffer containing ethidium bromide under ultraviolet irradiation. 16,17

Results and discussion

<em>P. aeruginosa</em> is commonly associated with hospital-acquired infections. With regard to the specimen site, of 530 samples, 150 (28.3%) were positive for <em>P. aeruginosa</em>, which was similar to results reported by Al-Haik et al 18 and Mansour et al 19 and fewer than Gad et al. 20 <em>P. aeruginosa</em> isolates were isolated from 65 of 332 (19.5%) wound swabs, 39 of 57 (68.4%) ear swabs, five of 26 (19.2%) burn swabs, six of 30 (20%) urine samples, eight of 12 (66.6%) sputum samples, eight of 35 (22.8%) stool samples, 19 of 38 (50%) of patients admitted to the intensive-care unit (ICU). Our results showed high incidence (68.4%) of <em>P. aeruginosa</em> among samples collected from patients suffering from otitis media, which was higher than reported by Umar et al, 21 who found that 23.2% of samples of otitis media were positive for <em>P. aeruginosa</em>. The distribution of isolates across major hospitals in Minia Governorate was analyzed. High incidence of <em>P. aeruginosa</em> was observed among samples collected from the chest hospital, while all samples obtained from Minia General Hospital were negative for <em>P. aeruginosa</em> (Figure 1).

<em>P. aeruginosa</em> possesses MDR against a wide variety of antibiotics. Resistance of <em>P. aeruginosa</em> is usually accompanied by the production of many BLs, active expulsion of antibiotics by efflux pump, and alteration of outer-membrane protein expression. 9,22 Resistance to variety of BLs is a growing problem, due to their continuous mutation, which makes BLs production the most common cause of DR and antimicrobial therapy failure. 23 Among BLs, ESBLs are widely distributed among Enterobacteriaceae members. They are also found in <em>Acinetobacter baumannii</em> and <em>P. aeruginosa</em>. At first, TEM-type ESBLs and SHV-type ESBLs were the most dominant among Gram negative isolates in Europe and other regions. Since the last decade, CTX-M type ESBL has became the most prevalent. ESBL production is widely spread among Enterobacteriaceae, especially <em>P. aeruginosa</em>. Our study showed that all <em>P. aeruginosa</em> isolates were completely resistant to azlocillin and amoxicillin–clavulanic acid. Of 150

| Gene          | Primers | Sequence                                      | Product size |
|---------------|---------|-----------------------------------------------|--------------|
| ESBL-CTX-M15  | CTX-M15-F, CTX-M15-R | 5'-CGTCACGCTGTGTTTAGAA-3' 75-ACGGCTTCTGGCTTAGTTT-3' | 780 bp       |
| bla<sub>VIM</sub> | VIM-F, VIM-R | 5'-GATGGTTTGGTACGTTGATGATG-3' 75-CAATGCAGGGACACCAG-3' | 390 bp       |
| bla<sub>IMP</sub> | IMP-F, IMP-R | 5'-GGAATAGGTGGGCTTAATTCTC-3' 75-CCAAACCACATACGTATCTT-3' | 188 bp       |
| bla<sub>GIM</sub> | GIM-F, GIM-R | 5'-TGCTACAACATTGTTGGCTCAAC-3' 75-AACCTGCTAGCAGCGGCA-3' | 477 bp       |
| bla<sub>SPM</sub> | SPM-F, SPM-R | 5'-AAAATCTGGTACCAAGCGGCGA-3' 75-AATTGGCTACGGAAGAAGA-3' | 271 bp       |

Abbreviations: ESBL, extended-spectrum β-lactamase; MBL, metallo-β-lactamase; IMP, imipenemase; VIM, Verona integron–encoded MBL; GIM, German imipenemase MBL; SPM, São Paulo MBL.
P. aeruginosa isolates, 100 (66.6%) were MDR and 21 (21%) of these were CRPA (eleven isolates were imipenem-resistant and ten meropenem-resistant). Figure 2 shows that 46%, 28.7%, and 28% of P. aeruginosa were resistant to polymyxin B, colistin sulfate, and tigecycline, respectively.

In this study, it was found that 54 (54%) isolates of MDR P. aeruginosa were ESBL producers. Similarly high production of ESBL was reported by Ahmad et al, who reported that ESBL production by P. aeruginosa isolates was 61.6%, while lower incidence (27.33%) was reported by Dutta et al. In addition, our results showed that eleven (11%) isolates were MBL-producing P. aeruginosa. Furthermore, MBL-producing strains represented 52.3% (eleven of 21) of CRPA isolates. Coexistence of ESBL and MBL was found among 5% of MDR P. aeruginosa and five of 21 (23.8%) CRPA isolates. Antibiotic-resistance patterns of ESBL-producing strains revealed that all ESBL producers were completely resistant to azlocillin, amoxicillin–clavulanic acid, ampicillin/sulbactam and cefepime. Co-resistance with other antibiotics was observed including colistin sulfate, tigecycline, and polymyxin B (Table 2). Also, MBL-producing strains showed high resistance to cefipime and carbenicillin (72.7% each), but lower resistance was observed against ciprofloxacin, colistin sulfate, and levofloxacin (36.3% each). Ilyas et al showed higher incidence of antibiotic resistance exhibited by MBL- and ESBL-producing P. aeruginosa. They reported that ESBL- and MBL-producing P. aeruginosa isolates were completely resistant to amoxicillin–clavulanic acid, ceftriaxone, ciprofloxacin, and cefepime. Also, they showed higher incidence of MBL-producing P. aeruginosa (25.7%) and lower incidence of ESBL production (8.5%). Mirsalehian et al found that all MBL-producing P. aeruginosa were colistin-sensitive and 37.5% were resistant to aztreonam, while in the present study low incidence of resistance to colistin, ciprofloxacin, and levofloxacin (36.4%) and a resistance rate of 54.5% were reported against aztreonam. Bashir et al reported that all MBL-producing P. aeruginosa isolates were resistant to gentamicin, ceftazidime, carbenicillin, tobramycin, ceftriaxone, ofloxacin, cefoperazone, cefoperazone–sulbactum, and ceftazidime–clavulanic acid and low resistance to polymyxin B.

The rapid spread and the emergence of MBL- and ESBL-producing P. aeruginosa isolated from hospitals is of great concern and threat. In addition, differences in resistance patterns among strains isolated from different countries may be attributed to antibiotic use, horizontal gene transfer, and environmental conditions. Therefore, it is important to test isolates for MBL and ESBL production and to test for antibiotic susceptibility before antimicrobial therapy.
Table 3 shows that the highest incidence of ESBL production was observed among MDR \textit{P. aeruginosa} samples isolated from ear infections (80%), followed by those isolated from chest infections (75%), and ICU patients (70%). The highest incidence of MBL production was observed among MDR \textit{P. aeruginosa} samples isolated from wound infections (19%) followed by those isolated from ear infections (14.3%). Nithyalakshmi et al\textsuperscript{29} reported that the frequency of occurrence of ESBL among \textit{P. aeruginosa} isolates was 21.96%, and most ESBL producers were obtained from urine samples (27.7%), followed by respiratory infection (23.68%), and wound infection (22.95%).

All MDR \textit{P. aeruginosa} isolates were tested for CTX-M15 and carbapenem-resistance genes: \textit{bla}\textsubscript{IMP}, \textit{bla}\textsubscript{VIM}, \textit{bla}\textsubscript{GIM}, and \textit{bla}\textsubscript{SPM}. It was found that 55.5% (30 of 54) of ESBL-producing \textit{P. aeruginosa} isolates were harboring CTX-M15, which was higher than another study\textsuperscript{17} reporting that out of 200 MDR \textit{P. aeruginosa} isolates, 19 were positive for CTX-M15, of which 64.28% were ESBL-positive. Although carbapenem resistance was found among 21 \textit{P. aeruginosa} isolates, only eleven were found to harbor MBL genes. Of 21 carbapenem-resistant strains, 42.8% (nine of 21) were positive for \textit{bla}\textsubscript{IMP}, 52.3% (eleven of 21) positive for \textit{bla}\textsubscript{VIM}, 52.3% (eleven of 21) positive for \textit{bla}\textsubscript{GIM}, and 38% (eight of 21) positive for \textit{bla}\textsubscript{SPM}.

The distribution of carbapenem-resistance genes and \textit{bla}\textsubscript{CTX-M15} among MDR \textit{P. aeruginosa}-producing ESBL and/or MBL isolates were tested (Table 4). Of eleven MBL-producing MDR \textit{P. aeruginosa}, three (27.2%) were CTX-M15, nine (81.8%) positive for \textit{bla}\textsubscript{IMP}, four (36.3%) for \textit{bla}\textsubscript{VIM}, five (45.4%) for \textit{bla}\textsubscript{SPM} and six (54.5%) for \textit{bla}\textsubscript{GIM}. Lower incidence was found by Zubair et al\textsuperscript{30} who reported that among 22 isolates positive for MBL production phenotypically, only five were harboring MBL genes. Furthermore, they reported that \textit{bla}\textsubscript{VIM} was the predominant gene, and none of the other genes were detected.

Also, It was found that 55.1% of ESBL/non-MBL-producing MDR \textit{P. aeruginosa} isolates were positive for

### Table 2 Antibiotic-resistance patterns of ESBL- and MBL-producing strains

| Antibiotic | ESBL-producing (n=54) | MBL-producing (n=11) |
|------------|------------------------|----------------------|
| Azlocillin  | 100                    | 100                  |
| Amoxicillin | 100                    | 100                  |
| clavulanic  | acid                   |                      |
| Ampicillin  | 100                    | 100                  |
| Sulbactam   |                        |                      |
| Tobramycin  | 81.5                   | 45.5                 |
| Aztreonam   | 83.3                   | 54.5                 |
| Cefepime    | 100                    | 72.7                 |
| Polymyxin B | 74.1                   | 54.5                 |
| Cefoperazone| 90.8                   | 54.5                 |
| Colistin sulfate | 37                    | 36.4                 |
| Levofloxacin| 55.6                   | 36.4                 |
| Amikacin    | 55.6                   | 63.6                 |
| Ciprofloxacin| 35.2                  | 36.4                 |
| Piperacillin| 51.9                   | 54.5                 |
| Gentamicin  | 46.3                   | 63.6                 |
| Imipenem    | 5.6                    | 100                  |
| Meropenem   | 7.4                    | 100                  |
| Carbenicillin| 46.3                  | 72.7                 |
| Tigecycline | 42.6                   | 45.5                 |

**Abbreviations**: ESBL, extended-spectrum \(\beta\)-lactamase; MBL, metallo-\(\beta\)-lactamase; \textit{P. aeruginosa}, \textit{Pseudomonas aeruginosa}.

### Table 3 Distribution of ESBL- and MBL-producing isolates among MDR \textit{Pseudomonas aeruginosa} isolates from different clinical specimens

| Type of infection    | MDR \textit{P. aeruginosa} | ESBLs | MBL |
|----------------------|-----------------------------|-------|-----|
|                      | n   | %   | n   | %   |
| Wound                | 59  | 40% | 4   | 19% |
| Ear                  | 20  | 80% | 3   | 14.3% |
| Burns                | 2   | 50% | —   | —   |
| Urinary tract        | 2   | 50% | —   | —   |
| Chest                | 4   | 75% | 1   | 4.8% |
| Gastroenteritis      | 3   | 66.7% | 1  | 4.8% |
| Patients admitted to ICU (from buccal cavity, skin swab, and eye swab) | 10  | 70% | 2   | 9.5% |
| Total                | 100 | 54% | 11  | 11% |

**Note**: Percentages correlated with number of MDR \textit{P. aeruginosa} isolates from each type of infection.

**Abbreviations**: ICU, intensive-care unit; MDR, multidrug-resistant; ESBL, extended-spectrum \(\beta\)-lactamase; MBL, metallo-\(\beta\)-lactamase.
CTX-M15, while none of these strains was found to harbor bla\textsubscript{IMP}. On the other hand, bla\textsubscript{VIM} was the most common carbapenem-resistance gene (14.2%). Rafiee et al\textsuperscript{31} and Laudy et al\textsuperscript{32} showed that all ESBL-producing isolates were negative for CTX-M gene, while Ahmed et al\textsuperscript{33} reported a lower incidence of bla\textsubscript{CTX-M} production (10.7%) among \textit{P. aeruginosa} strains isolated from Makkah hospitals. All MBL/non-ESBL-producing \textit{P. aeruginosa} harbored bla\textsubscript{IMP}-like genes and 50% were positive for bla\textsubscript{GIM}, while 33.3% only were positive for both bla\textsubscript{VIM} and bla\textsubscript{SPM} (Table 4). Similar findings were shown by Abiri et al\textsuperscript{34}. In contrast, Mirsalehian et al\textsuperscript{27} reported that bla\textsubscript{IMP} was the most prevalent carbapenemase gene among MBL-producing \textit{P. aeruginosa}, while 25% of MBL isolates were positive for bla\textsubscript{IMP} and all MBL isolates negative for bla\textsubscript{GIM} and bla\textsubscript{SPM}. Our results showed that five isolates of MDR \textit{P. aeruginosa} were ESBL and MBL coproducers. Three isolates (60%) were found to have bla\textsubscript{CTX-M15}, bla\textsubscript{IMP}, bla\textsubscript{GIM}, and bla\textsubscript{SPM}, and two (40%) were positive for bla\textsubscript{VIM}. MDR \textit{P. aeruginosa} samples were classified into seven groups according to the number of carbapenem-resistant genes harbored by MBL-producing \textit{P. aeruginosa} isolates, in order to study their demographic, phenotypic, and genotypic features: group A comprised MBL-producing \textit{P. aeruginosa} isolates harboring two genes (bla\textsubscript{IMP} and bla\textsubscript{GIM}), group B isolates positive for bla\textsubscript{IMP}, bla\textsubscript{VIM}, and bla\textsubscript{SPM}, group C including those which were positive for bla\textsubscript{IMP} group D isolates positive for bla\textsubscript{IMP} and bla\textsubscript{SPM}, group E isolates positive for bla\textsubscript{IMP}, bla\textsubscript{GIM}, and bla\textsubscript{SPM}, Group F isolates positive for bla\textsubscript{IMP} and bla\textsubscript{VIM}, group G MBL-producing \textit{P. aeruginosa} isolates positive for bla\textsubscript{VIM}, bla\textsubscript{GIM}, and bla\textsubscript{SPM}, and group H including isolates positive for bla\textsubscript{VIM} and bla\textsubscript{GIM} (Table 5).

Our study showed that all MBL-producing \textit{P. aeruginosa} isolates in groups A–H were obtained from Minia University Hospital except one isolate that had been obtained from a chest hospital. Of eleven MBL-producing \textit{P. aeruginosa}, five were ESBL producers and obtained from surgery and ICU units of Minia University Hospital. Of these, three (two from surgery unit and one from ICU) were positive for CTX-M15 gene. The isolate obtained from the ICU unit showed resistance to meropenem, polymyxin B, tigecycline, gentamicin, amikacin, and ceftazidime, which represents a great challenge for antimicrobial therapy patients. The other two isolates (surgery unit) showed resistance to gentamicin, ceftazidime, meropenem, imipenem, tigecycline, and colistin sulfate. Furthermore, the isolate obtained from the chest hospital belonged to group H, was positive for ESBL but negative for CTX-M15, and showed resistance to ceftazidime, cefoperazone, gentamicin, amikacin, tigecycline, polymyxin B, and meropenem. Chaudhary et al\textsuperscript{35} found that the frequency of bla\textsubscript{IMP} and bla\textsubscript{VIM} among MBL-producing strains was 28.73% and 47.12%, respectively. Coexistence of MBL and ESBL was found among 14.3% of isolates, of which 17.5% were positive for TEM and IMP genes and 14.8 positive for AMP-C and VIM. Also, they found that isolates coproducing ESBL and MBL were highly resistant to cefepim, piperacillin–tazobactam, ceftazidime, meropenem, and imipenem.

Our study showed the prevalence of ESBL- and MBL-producing \textit{P. aeruginosa} with limited sensitivity to antibiotics among the isolated strains, which indicates the great problem in the treatment of serious infectious diseases. In addition, there is a need to study resistance pattern of isolates and carry out screening for the presence of ESBL and MBL enzymes, in order to choose the proper antibiotic.

### Study limitations and future recommendations

We detected the distribution of genes only among resistant strains. Quantitative PCR assays are recommended for future studies, and should be performed to verify expression differences of different resistance genes in MDR \textit{P. aeruginosa}.

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*Table 4* Distribution of different groups of carbapenem-resistance genes in phenotypically positive ESBL- and MBL-producing \textit{Pseudomonas aeruginosa} isolates

|                | bla\textsubscript{CTXM-15} positive isolates | bla\textsubscript{IMP} | bla\textsubscript{VIM} | bla\textsubscript{GIM} | bla\textsubscript{SPM} |
|----------------|---------------------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
|                | n (%*)                                      | n (%*)                  | n (%*)                  | n (%*)                  | n (%*)                  |
| ESBL\textsuperscript{*}/MBL\textsuperscript{*}, n=49 | 27 (55.1)                                 | 0 (0)                   | 7 (14.2%)               | 3 (6.1)                 | 2(4)                    |
| ESBL\textsuperscript{*}/MBL\textsuperscript{*}, n=6 | 0 (0)                                      | 6 (100)                 | 2 (33.3)                | 3 (50)                  | 2 (33.3)                |
| MBL\textsuperscript{*}/ESBL\textsuperscript{*}, n=5 | 3 (60)                                     |                         | 2 (40)                  | 3 (60)                  | 3 (60)                  |

**Note:** *Percentages were correlated with number of isolates positive for ESBL, MBL, or both.

**Abbreviations:** ESBL, extended-spectrum β-lactamase; MBL, metallo-β-lactamase; IMP, imipenemase; VIM, Verona integron-encoded MBL; GIM, German imipenemase; MBL; SPM, São Paulo MBL.
## Table 5 Demographic, phenotypic and genotypic features of MBL-producing *Pseudomonas aeruginosa*

| Group | Carbapenem-resistance genes | Sample | Isolate source | Hospital         | ESBL production | bla<sub>CTXM15</sub> | Resistance pattern                  |
|-------|----------------------------|--------|----------------|------------------|----------------|---------------------|-------------------------------------|
| A     | +  -  +  -                | PA1    | ICU            | Minia University | – +            | – –                  | CAZ, CN, AK, PB, MEM                |
|       |                            | PA2    | Wound swab     |                  |                |                     | CAZ, CEP, TGC, MEM                 |
|       |                            | PA8    | ICU            |                  |                |                     | CAZ, CN, CT, CIP, IPM               |
| B     | +  +  –  +                | PA7    | Stool          | Minia University | –              | – –                  | CAZ, CEP, AK, CT, PB, MEM           |
| C     | +  –  –  –                | PA9    | Wound swab     | Minia University | +              | +                    | CAZ, CN, TGC, CIP, IPM              |
| D     | +  –  –  +                | PA11   | Ear discharge   | Minia University | – +            | – +                  | CAZ, CEP, AK, PB, CIP, IPM, CAZ, CEP, CN, CT, MEM |
|       |                            | PA33   | Wound swab     |                  |                |                     |                                     |
| E     | +  –  +  +                | PA13   | ICU            | Minia University | +              | +                    | CAZ, CN, AK, TGC, PB, MEM           |
| F     | +  +  –  –                | PA16   | Stool          | Minia University | –              | –                   | CAZ, CEP, AK, TGC, CIP, MEM         |
| G     | –  +  +  +                | PA19   | Wound swab     | Minia University | –              | –                   | CAZ, CN, AK, CT, PB, IPM            |
| H     | –  +  +  –                | PA27   | Sputum         | Minia University | +              | +                    | CAZ, CEP, CN, AK, TGC, PB, MEM      |

**Notes:** A, MBL-producing *P. aeruginosa* isolates positive for *bla<sub>IMP</sub>* and *bla<sub>CTX</sub>*; B, MBL-producing *P. aeruginosa* isolates positive for *bla<sub>IMP</sub>, bla<sub>CTX</sub>, and *bla<sub>VIM</sub>*; C, MBL-producing *P. aeruginosa* isolates positive for *bla<sub>IMP</sub>; D, MBL-producing *P. aeruginosa* isolates positive for *bla<sub>IMP</sub>, bla<sub>CTX</sub>, and *bla<sub>SPM</sub>*; E, MBL-producing *P. aeruginosa* isolates positive for *bla<sub>IMP</sub>, bla<sub>CTX</sub>, and *bla<sub>VIM</sub>*; F, MBL-producing *P. aeruginosa* isolates positive for *bla<sub>IMP</sub> and *bla<sub>SPM</sub>*; G, MBL-producing *P. aeruginosa* isolates positive for *bla<sub>VIM</sub>, bla<sub>CT</sub>, and *bla<sub>GIM</sub>*; H, MB-producing *P. aeruginosa* isolates positive for *bla<sub>IMP</sub> and *bla<sub>M</sub>*.

**Abbreviations:** ESBL, extended-spectrum β-lactamase; MBL, metallo-β-lactamase; ICU, intensive-care unit; CAZ, Ceftazidime, CN, Gentamicin, AK, Amikacin, PB, Polymyxin B, MEM, Meropenem, TGC, Tigecycline, CEP, Cefepime, CT, Colistin, CIP, Ciprofloxacin, IPM, Imipenem; IMP, Verona integron-encoded MBL; GIM, German imipenemase MBL; S>o Paulo MBL.
Conclusion
Using carbapenems in clinical practice was initially the solution to treatment of serious bacterial infections caused by β-lactam-resistant bacteria. Due to their widespread use, the emergence of MBL-producing strains and strains coproduce both ESBL and MBL was observed. As found in our study, strains showed high resistance to the commonly used antibiotics, which emphasizes the need to know the resistance patterns and testing for the coexistence of these enzymes, in order to design newer policies for antimicrobial chemotherapy.

Abbreviations list
MHT, modified Hodge Test; MDR, multidrug-resistant; ESBL, extended-spectrum β-lactamase; MBL, metallo-β-lactamase.

Disclosure
The authors report no conflicts of interest in this work.

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