Detection of metallo-\(\beta\)-lactamase genes in heteroresistant *Pseudomonas aeruginosa* isolated from clinical isolates in Egypt

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**ABSTRACT**
The aim of the study is to isolate and characterize *Pseudomonas aeruginosa* recovered from different clinical specimens and then study the susceptibility of the isolated strains to different antibiotics, screening for heteroresistant isolates and detecting the metallo-\(\beta\)-lactamase genes in these isolates. A total of two hundred and fifty clinical isolates were collected, from which one hundred forty five isolates revealed *Pseudomonas aeruginosa*. They were collected from different clinical specimens applied for bacteriological testing from hospitalized in-patients admitted to Kasr Al Aini Hospital and Al-Demerdash Hospital, Egypt, in the period from February 2016 to December 2017. Antimicrobial susceptibility testing was done using ten antibiotics. The study covered the heteroresistance of *P. aeruginosa* towards several classes of antibiotics to make the statistical analysis convenient and to overview the significance of this resistance. The minimum inhibitory concentration was detected for the heteroresistant *P. aeruginosa* resistant isolates. The polymerase chain reaction of the heteroresistant strains was performed to detect the metallo-\(\beta\)-lactamase genes SIM, IMP and SPM and then sequencing was done consequently. SIM, IMP and SPM metallo-\(\beta\)-lactam e genes were detected in the heteroresistant isolates. The isolates showed a high resistance pattern to ampicillin (98.62%) and a high sensitivity rate to imipenem (96.55%) and the IMP gene was the highly significant gene.

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porin proteins because they are small hydrophilic molecules (Dinesh et al., 2003). *Pseudomonas aeruginosa* has 163 familiar or estimated porin proteins lied on its genome sequence, in which 64 ones are placed as part of 3 families of porins the TonB-dependent gated porin family, the OprD-specific porin family and the OprM efflux family. The most popular channel in *Pseudomonas aeruginosa* for big substrates is known to be OprF, the resistance to imipenem is instable and Gram negative bacteria. They are found as group three enzymes because they have the ability to hydrolyse penicillins, cephalosporins and carbapenems, but are highly resistant mainly to all well known β-lactam antibiotics, while meropenem has a moderately higher effect on *Pseudomonas aeruginosa* (Ayalew et al., 2003). Carbenepens are sorted as a class of broad-spectrum β-lactam antibiotics, while meropenem has a moderately higher effect on *Pseudomonas aeruginosa* (Ayalew et al., 2003; Shah and Narang, 2005; Baldwin et al., 2008). The aminoglycosides have been advised to be used in the treatment of these infections and their mechanism of working is by suppressing the protein synthesis through binding to the 30S subunit of the ribosome (Hauser and Sriram, 2005).

The difficulties for the sensitivity of microbial identification methods is due to *Pseudomonas aeruginosa* adaptive ability and it is very important to develop systems with the ability to identify these bacteria since it has become necessary to develop genotype based characterization systems capable of accurately identifying these bacteria instead of any phenol-typic modifications, the molecular identification has finished the problem of different phenotype and allows for more accurate identification of bacteria (Drancourt et al., 2000). Heteroresistance is a type of resistance that seems to be a transitional state between resistance and sensitivity. Metallo etalactamases are sorted as group three enzymes because they have the ability to hydrolyse penicillins, cephalosporins and carbapenems, but are highly resistant mainly to all well known β-lactam inhibitors (Bush et al., 1995). These enzymes manufacturing is mainly constitutive (Majiduddin et al., 2002). They have identified Class B metallo etalactamases in both Gram positive and Gram negative bacteria. They are found in many clinically important species such as Acinetobacter spp. and *Pseudomonas aeruginosa*. The most popular Class B β-lactamases was belonging to the IMP and VIM families (Majiduddin et al., 2002). These enzymes are classified into three functional subgroups, B1, B2 and B3, depending on metal requirements. In subclass B1, it contains a lot of metallo etalactamases, it was revealed that Zn1 is coordinated very tightly and Zn2 is coordinated very loosely (Fabiane et al., 1998). The B3 subgroup contains a Zn1 site that binds zinc molecule very firmly (Valladares et al., 1997).

**MATERIALS AND METHODS**

The two hundred and fifty clinical specimens were submitted for bacteriological testing from hospitalized inpatients admitted to Kasr Al Aini Hospital (KAA) and Al-Demerdash Hospital (ADH), Egypt in the period from February 2016 till December 2017 and one hundred and forty five samples revealed *Pseudomonas aeruginosa*. The details of the collected isolates are shown in Table 1.

**Phenotypic methods**

**Isolates Culture and Identification**

The provided two hundred and fifty clinical isolates were subcultured on Nutrient Agar, 10% sheep blood agar and MacConkey’s agar supplied from (Oxoid Ltd, Basin Stoke, Hants, England). The clinical samples which confirmed *Pseudomonas aeruginosa* was preserved for further use by being subcultured weekly on MacConkey’s agar and stored for short period maintenance in the refrigerator at 4°C. Then the isolates were frozen at -80°C for long term use in glycerol medium. The one hundred and forty five *P. aeruginosa* isolates were identified using the conventional methods done by Monica Cheesbrough on the basis of Gram staining, motility, pigment production, catalase test, characteristic colony morphologies on MacConkey’s agar and oxidase reaction (Cheesbrough, 2006).

**Antimicrobial Susceptibility Testing (Disk Diffusion Method)**

The antimicrobial susceptibility testing of one hundred and forty five *Pseudomonas aeruginosa* strains were determined, according to Bauer and Kirby 1997. The following antibiotics were used, ampicillin 10 µg (AMP), ampicillin/sulbactam 20 µg (SAM), trimethoprim/sulphamethoxazole 25 µg (SXT), imipenen 10 µg (IPM), ciprofloxacin 5 µg (CIP), gentamicin 10 µg (CN), levofloxacin 5 µg (LEV), polymyxin B300 U (PB), ceftriaxone 30 µg (CRO30) and tetracycline TE 30 µg (TE). They were supplied from (HiMedia). The culture was done by making a saline suspension of the isolated colonies selected from an 18-24 hrs nutrient agar
plate. The zone diameter was recorded for the previously mentioned antibiotics and the results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI 2017) and then a screening of the heteroresistant strains of resistant P.aeruginosa was recorded.

**Determination of the antibiotics minimum inhibitory concentrations (MIC)**

Out of one hundred and forty five *P. aeruginosa* strains, forty three heteroresistant clinical isolates were determined from resistant ones for ampicillin, ampicillin/sulbactam, ceftriaxone, ciprofloxacin, gentamicin, levofloxacin, polymyxin B, sulphamthoxazole/trimethoprim and tetracycline powder supplied from (Himedia) using the agar dilution technique according to the procedure recommended by the NCLSI and the results were interrupted according to CLSI 2017 M100.

Each antibiotic was diluted two-fold dilution in Petri dishes using inoculums of $1 \times 10^8$ cfu/ml and incorporated into 19 ml of MHA to create a series of plates ranging from 64 to 0.015 mg/liter and the final inoculum chosen was 5 μl of a 1:25 dilution of the 0.5 McFarland standard suspensions. The plates were then incubated at 37°C for 24 hours. After incubation, the growth of microorganism is considered significant. Relationships at a p-value of less than or equal to 0.05 ($P < 0.05$) was considered statistically significant. Results were presented as percentages. Relationships at a p-value of less than or equal to 0.05 ($P < 0.05$) was considered statistically significant.

**Polymerase Chain Reaction to detect the metallo-β-lactamases genes in the heteroresistant isolates**

DNA isolation was done using (QIAmp DNA, England mini kit) according to manufacturer instructions, the following components were pipetted in a sterile clean PCR tube: 25 μl of 2X PCR Master Mix (Vivantis, Malayzia), 1 μl of Forward primer, 1 μl of Reverse primer, gemonic DNA with 100 ng concentration and nuclease free water up to 50 μl, the sample tubes were mixed gently, spun and placed in (BEOCO Germany thermal cycler). The primers were initially denaturated at 95°C for 1 minute, then annealing at 56°C for 45 seconds and extension at 72°C for 45 seconds for 35 cycles. The final extension was done at 72°C for 10 minutes for 1 cycle. The primers used were demonstrated in (Table 2). The agarose gel was prepared by melting 2 grams of agarose powder into 100 ml of 1X TAE electrophoresis buffer, then the ethedium bromide solution (0.5 μg/ml) was added to the melted agarose when the temperature was about 65°C and then mixed thoroughly. After that, the melted agarose was poured on the gel casting apparatus after proper installation of the desired comb, the gel was allowed to solidify at room temperature.

The electrophoresis chamber was filled with 1X TAE buffer. The PCR products (10 μl of PCR product+ 2 μl of loading dye) and molecular weight marker were loaded, the tank was then closed and the power supply was applied using 5 volts/cm of the tank length. Then the run was stopped when the bromphenol blue reached 2/3 of the gel length. The gel was visualized on a UV transilluminator and photographed using a camera.

**Sequencing**

The DNA sample was run on agarose gel electrophoresis to separate DNA fragments. Agarose gel band containing the desired DNA was cut and placed into a pre-weighed micro-centrifuge tube. The tube was centrifuged briefly to make sure the gel slice stayed at the bottom of the tube. Incubation at 50°C until the gel had melted completely. One gel volume of absolute ethanol was added to the solubilized sample and mixed thoroughly. The tubes were centrifuged at 10,000 xg for 1 min. The flow-through was discarded. Then in the column washing, the column was washed with 650 μl Wash Buffer and sample were centrifuged at 10,000 xg for 1 min.

The column was then dried by being centrifuged at 10,000 xg for 1 min to remove any residual ethanol. Finally, the column was placed into a clean micro-centrifuge tube for DNA elution. A 30-200 μl of Elution Buffer was added directly onto the column membrane and stood for 2 min. The tube was spun at 10,000xg for 1 min to elute DNA. DNA was stored at -20°C. Sequencing of the PCR products were done in ABI Prism Applied Biosystems HITACHI-3130XL Genetic Analyzer using BigDye Terminator v3.1 Cycle Sequencing Kit Applied Biosystems.

**Statistical Analysis**

Data generated in this study were analyzed using statistical software (SPSS version 20.0) for the test of significance. Results were presented as percentages. Relationships at a p-value of less than or equal to 0.05 ($P \leq 0.05$) was considered statistically significant.

**RESULTS AND DISCUSSION**

**Identification and confirmation of samples**

The one hundred forty five isolates revealed *P. aeruginosa* had shown positive morphological characters on MacConkey’s agar; Gram stain, motility, pigment production, catalase test and oxidase test.

**The Antimicrobial Susceptibility Testing using the KB method**

The susceptibility testing results and the antibi-
Table 1: The source, number, age range and gender of the collected samples

| Source         | Number of Samples | Age range          | Gender     | KAA         | ADH         |
|----------------|-------------------|--------------------|------------|-------------|-------------|
| Blood          | 15                | 23-65 years        | 12 Male    | 7 samples   | 8 samples   |
|                |                   |                    | 3 Female   |             |             |
| Cerebrospinal fluid | 31           | 23-81 years        | 20 Male    | 18 samples  | 13 samples  |
|                |                   |                    | 11 Female  |             |             |
| Ear washes     | 9                 | 13-59 years        | 4 Male     | 9 samples   |             |
|                |                   |                    | 5 Female   |             |             |
| Sputum         | 11                | 5-63 years         | 5 Male     | 7 samples   | 4 samples   |
|                |                   |                    | 6 Female   |             |             |
| Urine          | 55                | 2-76 years         | 35 Male    | 36 samples  | 19 samples  |
|                |                   |                    | 20 Female  |             |             |
| Wound          | 24                | 10-71 years        | 15 Male    | 9 samples   | 15 samples  |
|                |                   |                    | 9 Female   |             |             |

Table 2: Primers sequences

| Enzyme   | Primer | 5′3′ Sequence                      | Size bp |
|----------|--------|------------------------------------|---------|
| Class B  | IMP(1)R | 5′-GGAATAGAGTGCTTAAYTCTC-3′         | 232     |
|          |        | 5′-GGTTTAAYAAAACAACCACC-3′          |         |
|          | SPM(1)-F | 5′-AAAATCTGGGTACGCAAACG-3′           | 271     |
|          | SPM(1)-R | 5′-ACATTATCCGCTGGGAACAGG-3′          |         |
|          | SIM(1)-F | 5′-TACAAGGGATTCCGGATCG-3′            | 570     |
|          | SIM(1)-R | 5′-TAATGGCCTGTCCCCATGTG-3′           |         |

Figure 1: Antibiotic Sensitivity Pattern of Pseudomonas aeruginosa isolates (The x axis indicates the antibiotics used and the y axis indicates the strains)

Figure 2: blaSIM PCR assay (570 bp amplicon). M, 100 bp DNA ladder. 26 of the heteroresistant isolates were SIM positive

Figure 3: blaSIM PCR assay (570 bp amplicon). M, 100 bp DNA ladder. 26 of the heteroresistant isolates were SIM positive
| Antibiotics | CRO | SAM | GN | TE | SXT | IPM | AM | CIP | LEV | PB |
|-------------|-----|-----|----|----|-----|-----|----|-----|-----|----|
| P3          | 16  | 64  | 0.5| 64 | 32  | 1   | 64 | 0.25| 0.125| 2  |
| P11         | 2   | 64  | 32 | 64 | 64  | 32  | 64 | 8   | 16  | 32 |
| P15         | 8   | 32  | 8  | 64 | 64  | 16  | 32 | 16  | 32  | 64 |
| P16         | 32  | 64  | 32 | 64 | 64  | 0.5 | 64 | 0.25| 0.5  | 8  |
| P18         | 1   | 32  | 0.5| 64 | 32  | 1   | 32 | 0.5 | 2   | 16 |
| P21         | 16  | 64  | 2  | 32 | 32  | 0.125| 64 | 4   | 0.5  | 1  |
| P24         | 64  | 64  | 2  | 32 | 32  | 0.25| 32 | 0.5 | 0.125| 8  |
| P27         | 4   | 64  | 16 | 32 | 64  | 2   | 32 | 1   | 0.5  | 16 |
| P28         | 32  | 64  | 1  | 64 | 64  | 0.25| 32 | 0.25| 0.5  | 2  |
| P30         | 0.5 | 32  | 2  | 64 | 64  | 1   | 32 | 0.125| 0.25| 32 |
| P32         | 16  | 64  | 2  | 32 | 32  | 0.5 | 32 | 8   | 2   | 8  |
| P34         | 8   | 64  | 4  | 16 | 64  | 1   | 32 | 1   | 0.5  | 16 |
| P36         | 1   | 32  | 2  | 32 | 64  | 2   | 32 | 0.25| 0.5  | 8  |
| P43         | 2   | 4   | 2  | 1  | 64  | 0.5 | 32 | 0.25| 0.125| 4  |
| P46         | 32  | 32  | 8  | 16 | 64  | 1   | 64 | 1   | 0.5  | 16 |
| P50         | 4   | 64  | 2  | 16 | 32  | 1   | 32 | 32  | 8   | 2  |
| P52         | 1   | 64  | 2  | 16 | 64  | 2   | 64 | 1   | 0.5  | 16 |
| P61         | 0.5 | 64  | 1  | 32 | 64  | 2   | 32 | 0.25| 0.5  | 8  |
| P64         | 2   | 4   | 0.5| 2  | 64  | 0.5 | 32 | 0.25| 0.25 | 4  |
| P66         | 8   | 32  | 1  | 16 | 32  | 2   | 64 | 16  | 1   | 1  |
| P67         | 32  | 16  | 2  | 16 | 64  | 0.125| 64 | 0.5 | 1   | 8  |
| P74         | 4   | 32  | 1  | 32 | 64  | 2   | 32 | 1   | 0.5  | 16 |
| P75         | 1   | 32  | 2  | 64 | 64  | 0.5 | 64 | 1   | 1   | 32 |
| P78         | 32  | 32  | 1  | 32 | 32  | 0.25| 32 | 32  | 2   | 0.25|
| P80         | 8   | 64  | 2  | 16 | 64  | 1   | 64 | 1   | 0.5  | 64 |
| P83         | 16  | 32  | 1  | 32 | 64  | 0.5 | 32 | 0.25| 1    | 16 |
| P87         | 8   | 32  | 0.25| 16 | 32  | 2   | 32 | 16  | 0.125| 2  |
| P89         | 1   | 32  | 2  | 32 | 64  | 1   | 32 | 0.5 | 0.5  | 8  |
| P91         | 4   | 64  | 2  | 32 | 32  | 0.5 | 64 | 4   | 1   | 1  |
| P94         | 0.5 | 32  | 1  | 16 | 64  | 0.25| 32 | 1   | 0.5  | 16 |
| P96         | 32  | 32  | 0.5| 32 | 32  | 1   | 64 | 8   | 1   | 2  |
| P97         | 2   | 4   | 2  | 64 | 64  | 0.5 | 32 | 0.5 | 4   | 16 |
| P105        | 0.5 | 32  | 1  | 16 | 64  | 1   | 32 | 1   | 1   | 16 |
| P107        | 16  | 64  | 4  | 64 | 32  | 0.25| 32 | 8   | 1   | 0.5|
| P116        | 1   | 32  | 2  | 32 | 64  | 0.5 | 64 | 1   | 0.125| 8  |
| P118        | 8   | 32  | 8  | 32 | 64  | 1   | 32 | 0.5 | 16  | 32 |
| P122        | 0.5 | 32  | 0.5| 32 | 64  | 1   | 32 | 0.5 | 1   | 16 |
| P125        | 16  | 16  | 1  | 32 | 64  | 1   | 32 | 0.5 | 1   | 16 |
| P128        | 32  | 64  | 32 | 16 | 64  | 0.5 | 32 | 1   | 1   | 32 |
| P133        | 1   | 32  | 2  | 64 | 64  | 0.5 | 64 | 1   | 0.5  | 8  |
| P138        | 16  | 8   | 1  | 16 | 64  | 0.125| 64 | 1   | 0.25 | 16 |
| P141        | 32  | 32  | 4  | 16 | 64  | 0.25| 32 | 0.25| 0.125| 16 |
| P145        | 32  | 32  | 32 | 16 | 64  | 2   | 32 | 0.25| 0.5  | 16 |
high resistance pattern to ceftriaxone and ampicillin and lowest to levofloxacin and imipenem. This may be due to the hazardous use of ampicillin because they are well known between most of the people in Egypt and being highly used and recommended. Heteroresistance is a particular kind of bacterial resistance and the researcher’s concern increased greatly with this kind of resistance because it can lead to clinical detection error and an antifailure failure, heteroresistance detection was early studied on Staphylococcus aerus as first discovered methicillin-heteroresistant Staphylococcus aerus isolates taken from a patient who suffers from infectious disease (Chabbert, 1967).

Many countries until now have mentioned the vancomycin heteroresistant Staphylococcus aerus (Campanile et al., 2010). A certain recent study had revealed the Acinetobacter Baumannii to polymyxin B and carbapenem (Pournaras et al., 2005; Ikonomidis et al., 2009; Yau et al., 2009). Few researchers have proven heteroresistance of Pseudomonas aeruginosa to meropenem (Gordon and Wareham, 2009). In this study, we used KB antimicrobial susceptibility method and forty three samples showed heteroresistance. The heteroresistant isolates were very high to ampicillin, where thirty-six isolates revealed high heteroresistance to this antibiotic and heteroresistance to levofloxacin was only one isolate.

From the varying results of the heteroresistance, we observed that heteroresistance of Pseudomonas aeruginosa must be paid more attention in the future and most probably that the high resistance of Pseudomonas aeruginosa to most of carbapenems antibiotics is due to the presence of metallo-β-lactamases and the abnormal expression of efflux pump. The hazardous use of carbapenems antibiotics in Egypt may lead to high resistance. In another study of Pseudomonas aeruginosa to imipenem, they observed the sensitivity of one hundred and forty strains of Pseudomonas aeruginosa to imipenem using the KB method and VITEK method and our results agreed with them. In a certain study carried out in Sapien to determine the antimicrobial susceptibility testing of Pseudomonas aeruginosa isolated from children faces on different antibiotic, they revealed that the antimicrobial resistance pattern was as follows for ciprofloxacin (1%), imipenem (1%), gentamicin (3%), aztreonam (7%), cefepime (7%) and ceftazidime (8%) (Schelin et al., 2011). We agreed with the high susceptibility to imipenem, ciprofloxacin and gentamicin. Their results were reported to be contrasted with the multidrug resistant Pseudomonas aeruginosa detected in studies carried out with clinical strains (Balasubramanian et al., 2017).

In another study, they proved that combining these two mechanisms is the most likely explanation for the universal resistance of the isolates to tetracycline and chloramphenicol. Intrinsinc resistance of Pseudomonas aeruginosa to the folic acid synthesis inhibitor, co-trimoxazole, has been reported to its impermeability and the expression of the efflux pump MexAB-OprM (Köhler et al., 1996). They reported that all of the isolates were resistant to the aminoglycoside antibiotics (spectinomycin, paramomycin, kanamycin, neomycin and hygromycin B).

We have found that our results agreed with them. Future studies should be done to make effective combinations of the previously mentioned antibiotics to accelerate the recovery and shorten the duration of the illness period. To detect the imipenem heteroresistant strains, they used the double disk synergy test and SYBGreen RT-PCR for efflux pump (Huang et al., 1992; Pirnay et al., 2002), in their study, they confirmed twenty imipenem heteroresistant strains and demonstrated that the imipenem resistant mechanism of Pseudomonas aeruginosa is compromised with the high MexAB expression of efflux pump, Pseudomonas aeruginosa can go away from the bacterial membrane and resistance occur (Huang et al., 1992; Pirnay et al., 2002). They made PCR to detect the metallo-β-lactamases, but not detected in the twenty heteroresistant ones.

**Minimum Inhibitory Concentration**
MIC of antibiotics of heteroresistant *P. aeruginosa* clinical isolates were detected in (Table 3). The heteroresistant strains were forty three isolates and the test was done on all the used antibiotics and the results were interpreted from *Pseudomonas aeruginosa* non *Enterobacteriaceae*; (Zone Diameter Interpretative Standards and equivalent MIC Breakpoints) of the CLSI M100-2017 27th edition.

In this study, MIC was done on the heteroresistant *P. aeruginosa* resistant clinical isolates. It was reported that, for Ampicillin, all the forty three heteroresistant isolates were resistant to Ampicillin, showing MIC results ≥ 32 μg/ml. For Imipenem, forty one samples were sensitive, showing MIC results ≤ 2 μg/ml and two samples only were resistant, showing MIC results ≥ 8 μg/ml. MIC results.

In a certain study, they have observed the occurrence of multiple subpopulations and the MIC for the most highly resistant subclones, although, they have found that the heterogeneous sub populations had their resistance levels conferring a stable and the same expression of the heteroresistant phenotype to vancomycin in *Enterococci* (Alam et al., 2001). *P. aeruginosa* heterogeneously carbapenem has given a new protocol in the treatment to inhibit *P. aeruginosa* mutants resistance, optimizing the administration of meropenem has been recently reported (Tam et al., 2005).

**Polymerase Chain Reaction Results**

PCR was done for the heteroresistant clinical isolates, SIM gene was represented in (Figure 2). SPM gene was represented in (Figure 3) and the IMP gene was represented in (Figure 4).

In our study, PCR was done to detect the metallo-β-lactamases and we have found that the three genes were detected in the heteroresistant isolates and this confirmed the high resistance of the heteroresistance isolates to the penicillins. A certain study was carried out in Egypt revealed that 33 of 48 (68.7%) carbapenem resistant *P. aeruginosa* isolates produced MBL, their study demonstrated the presence of MBL producing *P. aeruginosa* was 82% (Diab et al., 2013). VIM-2 was the most prominent gene detected among the different MBL genes, their results were conferred to be the same as other results performed on previous studies to confirm that was VIM-2 the most frequently distributed MBL implicated in imipenem resistant *P. aeruginosa* (Walsh et al., 2008). In another Egyptian study, they reported the presence of 32.3% MBL (Mansour et al., 2013). In our study, we confirmed that the IMP gene was a highly significant gene. In another study that was done in Nepal to detect MBL genes, the VIM gene was the most frequently detected one (75%) and the IMP gene was (25%), their results agreed with previous studies in Kinea, revealing that the VIM gene is the most common MBL genes responsible for decreased susceptibility to imipenem (Pitout et al., 2008). In our study, we found IMP genes in twenty five isolates out of forty three heteroresistant ones (59%).

**Sequencing results**

**SIM gene sequences**

TACAAGGGATTCGGCATGTTAAAAAACAGAGCTTAA
GTAGTTCTTGCAATCAACAGCTATCTCATCGACAG
ACTCCAGCTTCCGAGGAGTACTGAAAGCTAGTGA
AATGTCGCTGAAAAATGTTTTCACTGTCAAATGGA
AGCATTTCAAACATTTCCAGGCACAGTACTGCT
GGATAGTGTCGTTTAATACAAAGTCCATCCCCACA
TATGCATCTAAATTGCAAAATGATTGCTAAATAAA
ATGGCAAATTCAACGGACACACTCCTTTTGAATAAG
AGACTTTTTGTTGGTCAAAAAATAAAATTGGAATTTT
TTATCCAGGCCGCAACAGACACTCAGATACAGAGAT
TGCTGAGTATCCATAAAAAATCTATTCGGGAGG
GTGGTTTAAAAACCCGATAGGTCGGTGCCATCAATG
AGCGAAAAATTGGAAAGCTTGGCGACACTCCGCAA
AAAAATGATATCAAAATACAGTAAGGCAAAACTTGTTATT
CCCAAGGCCACATGGAATCCGGAGACGCATCATT
GAACATCATGGGAAACAGGCCATTA

**SPM gene sequences**

AAAATCTGGGTACGCAAAGGCTTATGGATTG
GGCTAAGACTATGAAAGGCGAGAAAGTATGAGCC
ATCAAATACGCACTTTCATTGGAGCAGGGTG
GAAATGAAATTTACAAGAAGTAGGGCGCGGACAG
GTGGTGACGCATTGCAAAACAGTGTGACATT
GAGGAAAAACAGAAAGACCCGATAAAAAAGCGCTG
AGTTCTATAAAAAGGAGGATCTGAGCAAGGATT
CTGAGITCCCATCTGTTGACGGGATAAT

**IMP gene sequences**

Ggaatagagtggcttaattctcgatctatccccacgtactgctga
ttaaacaatgaactgttaaaaaagcggtaaggttagtcacacaa
ttcacctttgggagttatcgagttttagaaaaatgaagtt
TTTTTATCTCAGACCGGAGACCTAAGTAGGTTGG
GCTGTTTAAAAACCCGATAGGTCGGTGCCATCAATG
AGCGAAAAATTGGAAAGCTTGGCGACACTCCGCAA
AAAAATGATATCAAAATACAGTAAGGCAAAACTTGTTATT
CCCAAGGCCACATGGAATCCGGAGACGCATCATT
GAACATCATGGGAAACAGGCCATTA

**Statistical Analysis Results**

In this study, a screening of heteroresistance of resistant *P. aeruginosa* isolates was performed.
according to antimicrobial resistance profile and metallo-ß-lactamases. The distribution of heteroresistance in the antibiotics profile was determined and the results were convenient as the obtained results and imipenem will be recommended to be the drug of choice in the treatment of the heteroresistant strains of the resistant \textit{Pseudomonas aeruginosa}, followed by levofloxacin, then ciprofloxacin and finally gentamicin, the results were represented in (Figure 5).

\textbf{CONCLUSIONS}

The study of the sensitivity pattern of heteroresistant strains of resistant \textit{P. aeruginosa} against different antibiotics was detected and it was found that \textit{P. aeruginosa} isolates were highly sensitive to imipenem. We have found that the IMP metallo-ß-lactamase gene is a highly significant distributed gene.

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\textbf{Conflict of Interest}

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