



## IDENTIFICATION AND CHARACTERIZATION OF METALLO- $\beta$ -LACTAMASES PRODUCING *PSEUDOMONAS AERUGINOSA* CLINICAL ISOLATES IN AL-AZHER UNIVERSITY HOSPITAL, ASSIUT

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**Purpose:** Study aimed to determine the frequency of nosocomial infections caused by *P. aeruginosa* and its distribution among different intensive care units (ICUs), to determine the antibiotic resistance pattern, and to determine the prevalence of Metallo- $\beta$ -lactamase (M $\beta$ L) among isolates by phenotypic methods. **Methods:** A total of 74 *P. aeruginosa* isolates were identified from different clinical specimens in AL-Azher University Hospital. The antimicrobial susceptibility was tested by disk diffusion (Kirby-Bauer) method and phenotypic screening for M $\beta$ Ls was performed using Combined Disk Test (CDT) and double-disk synergy test (DDST). **Results:** The frequency of *P. aeruginosa* isolates from clinical specimens was 18% (74/412). The highest sensitivity was to imipenem 38 isolates (51.3%). The antibiotic sensitivity was in descending manner to meropenem (48.6%) > levofloxacin (28.4%) > ciprofloxacin and ceftazidime (21.6%), while the highest resistance rates were to carbenicillin 70 isolates (94.6%) then gentamicin (70.3%). Out of 74 *P. aeruginosa* isolates, 51/74 (69%) strains were multidrug resistant (MDR), based on the CDT results 26/74 (35.1%) isolates and by DDST 23/74 (31.1%) isolates were confirmed to be M $\beta$ Ls producers. **Conclusion:** There is a growing risk for isolation of MDR *P. aeruginosa* among the MBL-producer isolates suggests the need for continuous assessment of antimicrobial susceptibility and surveillance of antibiotic prescription. In addition, infection control measures are needed to prevent further dissemination of these organisms.

### INTRODUCTION

Infectious diseases are one of the leading causes of death worldwide due to the development of antimicrobial resistance<sup>1</sup>. *P. aeruginosa* is one of the most prevalent opportunistic human pathogen causing several

clinical infections including wound infection, pneumonia, urinary tract infections, endocarditis, meningitis, brain abscess, and bacteremia<sup>2&3</sup>. The increasing inappropriate use of broad-spectrum antibiotics has increased the emergence of multidrug resistant *P. aeruginosa* (MDRPA) isolates which complicates the

process of therapy and limits treatment options<sup>4</sup>. Multidrug resistant is defined as being resistant to at least 3 anti-pseudomonal antibiotic-groups including  $\beta$ -lactams, aminoglycosides, and fluoroquinolones<sup>5</sup>. The growing threat of antimicrobial resistance in *P. aeruginosa* relies on one hand in the extraordinary capacity of this microorganism for developing resistance to almost any available antibiotic by the selection of mutations in chromosomal genes, and on the other, in the increasing prevalence of transferable resistance determinants, particularly those encoding class B carbapenemases (or metallo- $\beta$ -lactamases (M $\beta$ L)<sup>6</sup>. Metallo- $\beta$ -lactamase (M $\beta$ L) production is increasingly reported as a cause of high-level carbapenem resistance among *P. aeruginosa*, an important nosocomial pathogen that is notorious for multi-drug resistance<sup>7</sup>. Genes encoding M $\beta$ Ls are generally located within class 1 integrons together with those encoding aminoglycoside modifying enzymes, which therefore confer MDR<sup>8</sup>.

## PATIENTS AND METHODS

In this study, seventy four *P. aeruginosa* isolates were obtained from clinical specimens submitted for bacteriological testing from hospitalized in-patients admitted to AL-Azher University Hospital, and Molecular Biology Research Unit, Assiut University, Egypt during the period between November 2015 to April 2017.

### Isolation and identification of *P. aeruginosa* isolates

These isolates were identified by standard laboratory methods including bacteriologic and biochemical methods such as; Gram staining, colony morphologies on MacConkey's agar, biochemical tests such as oxidase, catalase, Oxidative-fermentative test, growth on media such as Triple Sugar Iron Test (TSI), cetrimide agar and growth at 42°C. These isolates were stored at -70°C in trypticase soy broth containing 20% glycerol and sub cultured twice prior to testing.

### Antimicrobial susceptibility testing

The susceptibility pattern of isolates to different antibiotics were examined using disk diffusion method (Kirby-Bauer) on Muller-Hinton agar plates according to Clinical and Laboratory Standards Institute (CLSI)<sup>9</sup>. The antimicrobial disks were included: imipenem (10  $\mu$ g), meropenem (10  $\mu$ g), ceftazidime (30  $\mu$ g), carbenicillin (100  $\mu$ g), amikacin (30  $\mu$ g), gentamicin (10  $\mu$ g), Ciprofloxacin (5  $\mu$ g), Levofloxacin (5  $\mu$ g). inoculated plates and incubated overnight. The zones of inhibition were measured and interpreted.

### MIC Determination for *P. aeruginosa* isolates by Epsilon meter test (E test)

Determination of MIC *P. aeruginosa* isolates by Imipenem (IP)-E test and Meropenem (MP)-E test (bioMérieux SA, Marcy l'Etoile, France). Results were interpreted using CLSI (2016)<sup>10</sup> breakpoints. The breakpoints for imipenem and meropenem were as follows: susceptible (S)  $\leq 4$   $\mu$ g/ml and resistant (R)  $\geq 16$   $\mu$ g/ml.

### MBL screening and confirmation by phenotypic methods

Combination disk diffusion test (CDDT) was used for phenotypic detection of MBLs producing *P. aeruginosa* strains. In brief a 0.5 M EDTA solution was prepared and then was added to 10  $\mu$ g imipenem and meropenem disks to obtain a concentration of 750  $\mu$ g. The IPM and IPM -EDTA disks were placed on the plate which were inoculated with *P. aeruginosa*. The inhibition zones of these disks with and without EDTA solution were compared after 16–18 hrs of incubation in air at 35°C. An increase of  $\geq 7$  in the zone diameter for IPM in the presence of EDTA was considered as positive result.

Double-disk synergy test (DDST), in brief a 10  $\mu$ l of 0.5 M (750  $\mu$ g) EDTA solution was added to filter paper blank disc, was placed on the surface of the agar plate. IPM disc (10  $\mu$ g) or MEM disc (10  $\mu$ g) was placed 13 mm center to center from the EDTA disc. The plates were incubated for 16-18 hrs at 35°C. Enhancement of the inhibition zone in the area between the imipenem (or meropenem) disc and the EDTA disc in comparison with the inhibition zone on the far side of the drug was interpreted as positive for M $\beta$ L production<sup>11</sup>.

## RESULTS AND DISCUSSION

### Results

Isolation and Identification of pathogens were done on specimens according to standard procedures. 412 isolates from different clinical samples were collected. Out of 412 isolates, *Enterobacteriaceae sp.* were the most common isolates detected, they were 226 (54.85%). The incidence of other microorganisms was *P. aeruginosa* 74 (17.96%), *Staphylococci sp.* 98 (23.79%) and *Candida sp.* 14 (3.4%). Microbial isolates are shown in table 1.

**Table 1:** Distribution of isolated pathogens among total 412 clinical specimens.

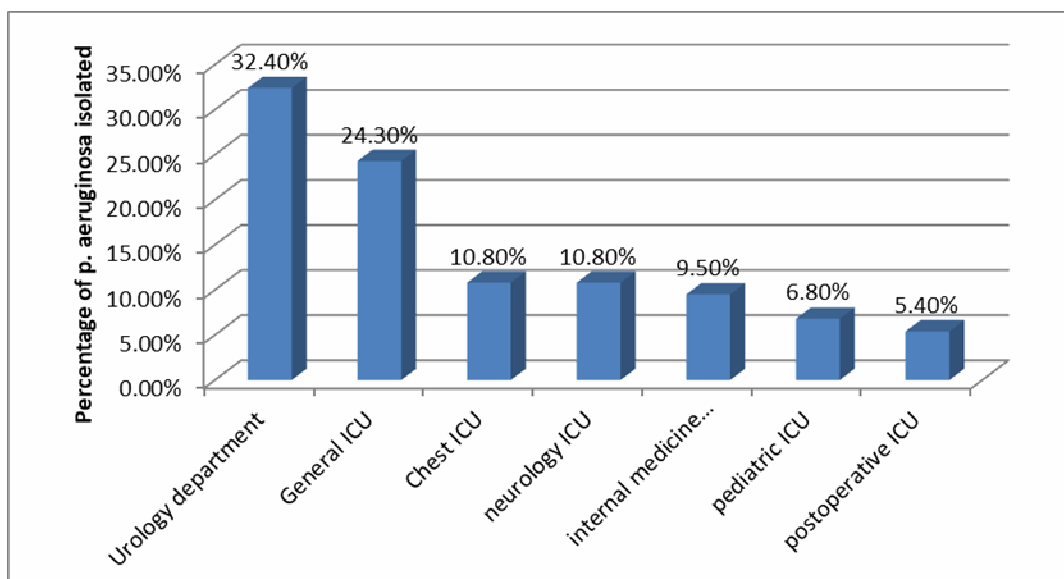
Type	No. of isolates	Percentage
<i>P.aeruginosa</i>	74	17.96%
<i>Enterobacteriaceae sp.</i>	226	54.85%
<i>Staphylococci sp.</i>	98	23.79%
<i>Candida sp.</i>	14	3.4%
Total	412	100%

Distribution of *P. aeruginosa* isolates among different wards/ICUs. *P. aeruginosa* isolates were mostly isolated from Urology

department representing 32.4% (24/74) from total *P. aeruginosa* isolates, followed by General ICU representing 24.3% (18/74), Chest ICU and neurology ICU representing 10.8% (8/74), internal medicine ICU 9.5% (7/74), pediatric ICU 6.8% (5/74), and then postoperative ICU representing 5.4% (4/74) are shown in figure 1.

**Antimicrobial susceptibility.** The highest sensitivity was to imipenem 38 isolates (51.3%). The antibiotic sensitivity then decrease in descending manner to be meropenem (48.6%) > levofloxacin (28.4%) > ciprofloxacin and ceftazidime (21.6%) while the highest resistance rates were to carbenicillin 70 isolates (94.6%) then gentamicin (70.3%). The rate of resistance to antibiotics is shown in the table (2). Out of 74 *P. aeruginosa* isolates, 51 (69%) strains were MDR and 23 (32%) strains were non-MDR strains.

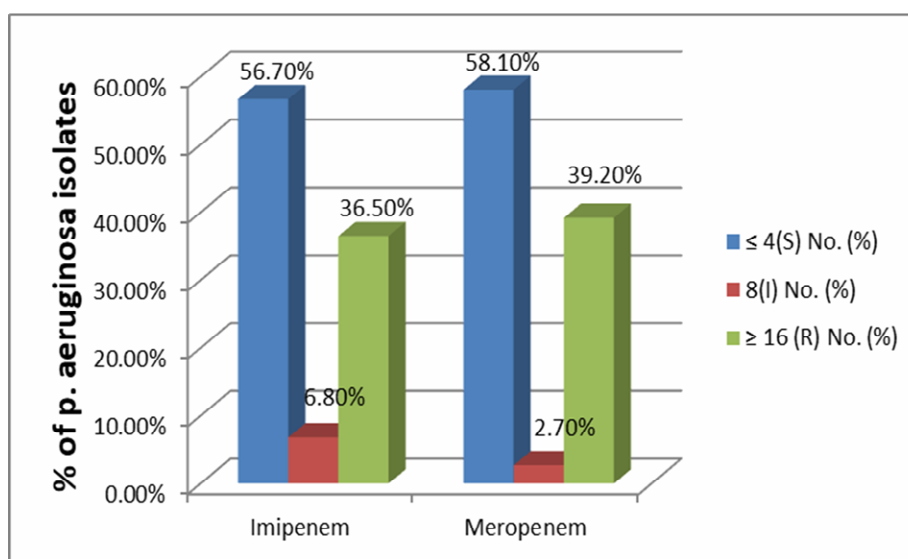
**Determination for *P. aeruginosa* isolates.** All *P. aeruginosa* isolates (74 isolates) were tested for accurate determination of their MIC using the Imipenem (IP)-E test and Meropenem (MP)-E test strips. Results were interpreted using the breakpoints of CLSI (2016) as shown in figure 2.



**Fig. 1:** Distribution of *P. aeruginosa* isolates among different wards/ICUs.

**Table 2:** Antimicrobial susceptibility pattern of 74 *P. aeruginosa* urine isolates.

Antimicrobial drug	No. of resistant isolates (%)	No. of intermediate isolates (%)	No. of sensitive isolates (%)
Imipenem	27 (36.5%)	9 (12.2%)	38 (51.3%)
Meropenem	29 (39.2%)	9 (12.2%)	36 (48.6%)
Ceftazidime	49 (66.2%)	9 (12.2%)	16 (21.6%)
Amikacin	43 (58.1%)	19 (25.7%)	12 (16.2%)
Gentamicin	52 (70.3%)	9 (12.2%)	13 (17.5%)
Ciprofloxacin	51 (68.9%)	7 (9.5%)	16 (21.6%)
Levofloxacin	46 (62.1%)	7 (9.5%)	21 (28.4%)
Carbenicillin	70 (94.6%)	1 (1.4%)	3 (4%)

**Fig. 2:** Results of MIC of Imipenem and Meropenem using (IP)-E test and (MP)-E test strips in 74 *P. aeruginosa* isolates.

**Detection of MBL by CD.** Out of the 74 *P. aeruginosa* isolates, 26 (35.1%) and 25 isolates (33.8%) showed an increase in the zone of inhibition of about 7 mm or more around IPM-EDTA and MEM-EDTA disks compared to IPM and MEM disk alone (positive for MBLs production), respectively are shown in the figure 3 and table 3.

**Detection of MBL by DDST.** Out of the total 74 *P. aeruginosa* isolates, 23 (31.1%) and 21 isolates (28.4%) showed positive results for MBLs production using IPM and MEM, respectively, are shown in the figure 4 and table 4. but all IP and MP E-test susceptible *P.*

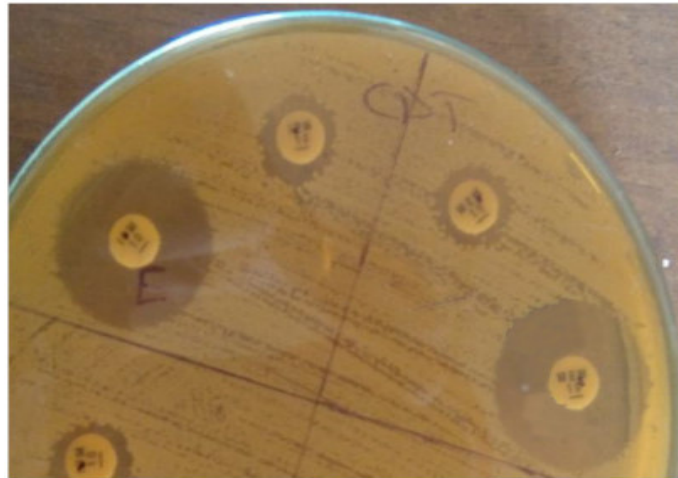
*aeruginosa* isolates showed negative results of DDST.

### Discussion

This study included patients suffering from nosocomial UTIs over a period of 17 months from November 2015 to April 2017. The aerobic pathogens causing UTI were identified. The antibiotic resistance pattern, for *P. aeruginosa* isolates was performed, the frequency of multidrug resistance among nosocomial *P. aeruginosa* infections, and carbapenems antibiotic sensitivity pattern of *P. aeruginosa* isolates were determined. Phenotypic detection of Metallo-β-lactamase production.

**Table 3:** Results of CD test of all 74 *P. aeruginosa* isolates using IPM (10 µg) and MEM (10 µg) for MβL detection.

Sample	CDT using IPM		CDT using MPM	
	Positive	Negative	Positive	Negative
	No. (%)	No. (%)	No. (%)	No. (%)
Total No of <i>P. aeruginosa</i> isolates (n= 74)	26 (35.1%)	48 (64.9%)	25 (33.8%)	49 (66.2%)



**Fig. 3:** Detection of MβL in carbapenem resistant *P. aeruginosa* isolate by CD test (+Ve).

**Table 4:** *P. aeruginosa* isolates using IPM (10 µg) and MEM (10 µg) for MβL detection.

Sample	DDST using IPM		DDST using MPM	
	Positive	Negative	Positive	Negative
	No. (%)	No. (%)	No. (%)	No. (%)
Total No of <i>P. aeruginosa</i> isolates (n= 74)	23 (31.1%)	51 (68.1%)	21 (28.4%)	53 (71.6%)



**Fig. 4:** Detection of MβL in carbapenem resistant *P. aeruginosa* isolate by DDST (+Ve).

During the study period, rate of isolation of nosocomial *P. aeruginosa* urine isolates was 17.96% of isolated uropathogens. This finding agreed to some extent with that reported by Gad *et al.*<sup>12</sup> who identified *P. aeruginosa* isolates in 18.2% of the clinical samples and Wassef *et al.*<sup>13</sup> who identified *P. aeruginosa* isolates in 20.7% of different clinical samples, This rate were much higher than that reported by Aminizadeh and Kashi<sup>14</sup>, where the rate of incidence of *P. aeruginosa* in UTI was 13.2% In Malaysia, also Abbas *et al.*<sup>15</sup> who identified *P. aeruginosa* isolates in (12.5%) of the clinical samples and Mohammadi *et al.*<sup>16</sup> who identified *P. aeruginosa* isolates in (12.35%) of different clinical samples. However, these results were less than those found by Mansour *et al.*<sup>17</sup>, who reported that the rate of isolation of *Ps. aeruginosa* from patient samples In Egypt and Saudi Arabia, was 32.8% and 30.0% respectively.

The leading uropathogens in this study were *Enterobacteriaceae sp.* (54.85%). This was comparable to that reported by in Sudan reported by Shareef and Yagoub<sup>18</sup> (68.01%) and to Milan and Ivan<sup>19</sup> in Serbia (64%). There were much higher rates reported in Nepal and Pakistan by Chandrashekhar *et al.*<sup>20</sup> and Khan *et al.*<sup>21</sup>. On the other hand rates ranging from (25) to (37.4%) were reported in Japan and Turkey by Shigemura *et al.*<sup>22</sup> and Savas *et al.*<sup>23</sup>

In this study *Candida sp.* forms (3.4%) of isolated pathogens. This rate was more or less similar to (3.7%) found in India by Hasan *et al.*<sup>24</sup>, lower than reported in Kuwait (16.5%) by Al Benwan *et al.*<sup>25</sup>. In Saudi Arabia, the frequency of nosocomial uro-pathogens isolates was studied by Alzohairy and Khadri<sup>26</sup>, where they showed that *E. coli* was the predominant pathogen and *Klebsiella spp.* were the second common organism in hospital-acquired infection followed by *P. aeruginosa* strains.

In our study, the highest number of *P. aeruginosa* strains were recovered from Urology department followed by General ICU then chest ICU and neurology ICU with percentages of 32.4% (24/74), 24.3% (18/74), 10.8% (8/74) and 10.8% (8/74), respectively (Fig. 1). These findings disagree to some extent with those of Javanbakht *et al.*<sup>27</sup> who reported

that Postoperative ICU were the most frequent category of infection (46.5%) in Imam Reza hospital, followed by Urology department (22.7%), and General ICU (9%). These differences may be due to difference in the number of patients, place of study and genetic susceptibility.

The present study revealed that the resistance rates of *P. aeruginosa* were ranging from very high (94.6%) to carbenicillin to moderate to ceftazidime (66.2%) and high incidence of resistance can explain by our routine use of the drug (Table 2). These high values of resistance which were observed were comparable to those of the reports from Gujarat, with a resistance value of (75%)<sup>28</sup>. Also, our results were consistent with data of Fazlul *et al.*<sup>29</sup>, who stated that nosocomial *P. aeruginosa* urine isolates were highly resistant to ceftazidime (61.9%).

In our study, the rate of resistance of *P. aeruginosa* urine isolates to imipenem was (36.5%) and to meropenem was (39.2%) in the same table. Our results were identical with those reported by Ashour and El-Sharif<sup>30</sup> in Egypt who concluded that *Acinetobacter* and *Pseudomonas* species exhibited the highest resistance levels to imipenem (37.03%) among other Gram-negative organisms.

Our results were higher than those reported by Wang *et al.*<sup>31</sup> documented low rate of imipenem and meropenem-resistance among *P. aeruginosa*, it was (13%) and (16%) respectively. Queenan *et al.*<sup>32</sup> stated that rate of imipenem and meropenem resistance among *P. aeruginosa* isolated from patients with UTIs was (18%) and (22%), respectively. Resistance rates to carbapenems may vary and depends on local antibiotic policies, origin of the strains, and geographic location<sup>33</sup>.

Our study showed marked increase in imipenem resistance (39.2%) which may be attributed to increased use of imipenem in the hospital. This rate of carbapenem resistance reflects a threat limiting the treatment options in our hospitals. This can be explained in part by the increase in consumption of antimicrobial agents in the last decade leading to a selective pressure of antibiotics on *P. aeruginosa* and consequently the bacteria modify the resistant mechanisms. A similar high rate of resistance

has been reported in many developing countries worldwide<sup>34</sup>.

The resistance rate of *P. aeruginosa* to gentamicin was (70.3%) and amikacin was (58.1%). In agreement with our result, Fazlul *et al.*<sup>29</sup>, observed that the resistance of nosocomial *P. aeruginosa* urine isolates to gentamicin and amikacin was (75%) and (48%) respectively. Ramirez and Tolmasky<sup>35</sup> reported much high rate of resistance to gentamicin (81%). Sibi *et al.*<sup>36</sup> found that resistance of *P. aeruginosa* to gentamicin and amikacin was (79%) and (51%), respectively. Zhao *et al.*<sup>37</sup> found that the resistance rate of *P. aeruginosa* to gentamicin and amikacin was (77%) and (61%), respectively.

Fluoroquinolones are most commonly used in urinary tract infections. But resistance to fluoroquinolones is increasing. The resistance rate to ciprofloxacin was (68.9%) and levofloxacin was (62.2%). These results were similar to those reported by Gad *et al.*<sup>12</sup>, who found that most of the *P. aeruginosa* isolates were resistant to both levofloxacin (64%), and ciprofloxacin (62%). Abbas *et al.*<sup>15</sup> also found that most of the *P. aeruginosa* isolates were resistant to levofloxacin (69.4%), and ciprofloxacin (67.1%). These results disagreed with the study of Kobayashi *et al.*<sup>38</sup> also found that (22.4%) of the *P. aeruginosa* isolates were resistant to ciprofloxacin. These rates were lower than that reported by Manjunath *et al.*<sup>39</sup> in India where the rate of ciprofloxacin resistance was (85%).

In our study, the prevalence of MDR strains among *P. aeruginosa* urine isolates was 51 (69%). Our result was coincides with studies that were done by Mohanasoundaram<sup>40</sup> who found that, the percentage of the MDR in the *P. aeruginosa* strains had increased from (64%) in 2008 to (71%) in 2010; Alzohairy and Khadri<sup>26</sup> found that, *P. aeruginosa* (58.3%) was the most common MDR uro-pathogens followed by *E. faecalis* (55.5%) and *E. coli* (53.6%). Our results was higher than Kirikae *et al.*<sup>41</sup> who reported that the percentage of MDR-*P. aeruginosa* isolates in the urinary tract was significantly greater in both medical facilities and clinical laboratories, where the percentage of MDR strains was (43.8%) and (41.6%),

respectively. However, the result was lower than (76%) reported by El-Shouny *et al.*<sup>42</sup>.

In this study, phenotypic detection of carbapenemase producing *P. aeruginosa* isolates was done by determination of MICs of carbapenems using E-test. IP & MP E-test identified 27 carbapenemase-producing *P. aeruginosa* isolates. IP E-test results showed that 36.5% (27/74) of *P. aeruginosa* isolates were imipenem resistant (MIC above 16 µg/ml), while 6.8% (5/74) of the isolates showed imipenem intermediate susceptibility (MIC 8 µg/ml) and 56.7% (42/74) of the isolates were imipenem sensitive (MIC below 4 µg/ml) (Fig. 2). These results were similar to those reported by Arora *et al.*<sup>43</sup> who found that rate of imipenem-resistant among *P. aeruginosa* urine isolates was (37%). In the Middle East the occurrence of imipenem resistant *P. aeruginosa* is alarmingly recognized. In Saudi Arabia, the resistance rate of *P. aeruginosa* to imipenem was increased to (38.57%) in 2011<sup>44</sup>.

MP E-test results showed that 39.2% (29/74) of *P. aeruginosa* isolates were meropenem resistant (MIC above 16 µg/ml), while 2.7% (2/74) of the isolates showed meropenem intermediate susceptibility (MIC 8 µg/ml) and 58.1% (43/74) of the isolates were meropenem sensitive (MIC below 4 µg/ml). In agreement with our results, a study in Egypt reported a resistance rate of (37.7%) to meropenem among *P. aeruginosa* isolated from hospitalized cancer patients<sup>45</sup>. This is explained by the differences in the pattern of antibiotic prescription and usage between the two studies.

In our study, CDT and DDST were done as phenotypic methods for detection of MβL-producing *P. aeruginosa* isolates including both carbapenem susceptible and non-susceptible strains. By CDT, 35.1% (26/74) and 33.8% (25/74) of *P. aeruginosa* isolates were MβL producers phenotypically by IPM/EDTA and MEM/EDTA combined disks, respectively (Table 3). These findings are similar to high extent to the prevalence of MBL producers in Egyptian study which was (32.3%) by Mansour *et al.*<sup>17</sup>, from UK (38.3%) by Ellington *et al.*<sup>46</sup>.

Regarding the DDST, 31.1% (23/74) and 28.4% (21/74) of *P. aeruginosa* isolates were

MβL producers phenotypically by IPM and MEM, respectively (Table 4). These results are in accordance with those of other studies in Egypt by Mansour *et al.*<sup>17</sup> who reported that the prevalence of MβL producers in Egyptian study was (32.3%). In 2012, Polotto *et al.*<sup>47</sup> surveyed 56 *P. aeruginosa* isolates by disk diffusion method and showed that 54/56 (96.4%) of isolates were resistant to imipenem. They also showed that 17 /56 (30.3%) of imipenem-resistant strains were positive for production of MBL.

### Conclusion

This study shows that there is increasing prevalence of β-lactamase producing *P. aeruginosa* in nosocomial infections in AL-Azher University Hospital. This is a major cause of concern as this implies that more and more instances of multidrug resistance are emerging. This leads to an overall negative impact on the health concerns and amounts to increasing difficulty in combating disease. It is recommended that awareness about antibiotic use and abuse be made a priority and measures of curbing unchecked use of prescription antibiotics be put into place. Establishment of infection control programs will help to lower the incidence of resistance in *P. aeruginosa*.

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## نشرة العلوم الصيدلانية جامعة أسيوط



### تحديد وتوصيف الميتالوبيبتالاكتاميز المنتج من عزلات ميكروبات السيدوموناس أيروجينوزا السريرية في المستشفى الأزهر الجامعي

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هدفت هذه الدراسة إلى تحديد نسبة انتشار العدوي بيكتريا السودومونس ايرجينوزا في مرضي العدوى المكتسبة في وحدات العناية المركزة المختلفة وقسم المسالك البولية في مستشفى الأزهر الجامعي بأسيوط ، تم عزل (٧٦) عزلة من ميكروب السودومونس ايرجينوزا بنسبة ١٨% من الاجمالي الكلي للميكروبات المعزولة المختلفة (٦٢٩) ، وكانت أعلى نسبة من السودومونس ايرجينوزا تم العثور عليها في قسم المسالك البولية (٣٢,٤%) وحدة العناية المركزة عامة (٢٤,٣%) ، تليها كل من وحدة العناية المركزة الصدر الباطني ووحدة العناية المركزة العصبية بنسبة (١٠,٨%) ، ثم وحدة العناية المركزة الباطني (٩,٥%) ، وحدة العناية المركزة الباطني أطفال (٦,٨%) واخيرا وحدة العناية المركزة بعد العمليات (٥,٤%).

تم دراسة حساسية السودومونس ايرجينوزا المعزولة للمضادات الحيوية المختلفة بواسطة طريقه الانتشار القرصي ، أظهر الاختبار أن ٧٠ (٩٤%) كانت مقاومة للكاربينيسيلين ، ٥٢ (٧٠%) مقاومة للجنتاميسين ، ٥١ (٦٨%) مقاومة لكل من سيبروفلوكساسين ، ٤٩ (٦٦%) مقاومة لسيفتازديم ، ٤٦ (٦٢%) مقاومة لليفلوكساسين ، ٤٣ (٥٨%) مقاومة لأميكاسين ، ٢٩ (٣٩%) مقاومة للميروبيديم ، ٢٧ (٣٦%) مقاومة للايمبيديم و٦٩% من المعزولات كانت مقاومة لأدوية متعددة ، كما وضحت الدراسة أن نسبة السلالات المتعددة المقاومة لأكثر من ثلاث مضادات ميكروبية كانت تمثل ٦٩% ، ونسبة السلالات الغير متعددة المقاومة للمضادات الحيوية كانت تمثل ٣١%.

تم تحديد ادني تركيز مثبت MIC للمضاد الحيوي للايمبيديم والميروبيديم بواسطة اختبار MP-E و IP-E. أظهرت نتائج اختبار IP-E أن ٣٦,٥% من سلالات السودومونس ايرجينوزا مقاومة للايمبيديم ، ونتائج اختبار MP-E. أن ٣٩,٢% من سلالات السودومونس ايرجينوزا مقاومة للميروبيديم.

وقد تم ايضا الكشف الظاهري لانزيمات الميتالو-بيتا-لاكتاميز في جميع معزولات السودومونس ايرجينوزا بعدة طرق ظاهرية مختلفة منها: اختبار القرص المشترك واختبار القرص مزدوج التآزر باستخدام قرص المضادات الحيوية ايمبيديم وميروبيديم ذات التركيز ١٠ ميكروجرام.