Phenotypic Detection of Metallo-β-Lactamases and Extended Spectrum β-Lactamases Among Gram Negative Bacterial Clinical Isolates

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Detection of metallo-β-lactamases (MBLs) and extended spectrum-β-lactamases (ESBLs) among Gram negative bacilli (GNB) is crucial for the optimal treatment of patients and to control spread of resistance. However, NCCLS documents do not contain a method for detection of MBL producing isolates. Lack of sufficient reports from Egypt indicated the need for this study to determine the proportion of MBL producers among GNB isolated from clinical multi-drug resistant (MDR) pathogens. We also attempted to assess the efficiency of several phenotypic tests for the rapid and convenient detection of MBLs among *Pseudomonas* and *Acinetobacter spp.* The efficiency of testing ceftazidime (CAZ) resistant versus imipenem (IMP) resistant pathogens was also compared. A total of 70 CAZ intermediate/resistant GNB were identified and tested for antibiotic sensitivity by Vitek 2 Automated System (bioMérieux). Screening for ESBLs was performed by Oxoid combined test (CD02) and confirmed by Vitek 2. The phenotypic detection of MBL production among *Pseudomonas* and *Acinetobacter* isolates was performed by modified Hodge test, EDTA-disk synergy test (EDST), IMP-EDTA combined disk test (CDT) and E-test MBL strips. Negative control strain (*P. aeruginosa* ATCC 27853) was included in the tests. Of the 70 GNB pathogens, 25(35.7%) were ESBL producers mainly *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*), while 8(11.4%) *P. aeruginosa* isolates were IMP resistant. Their MIC was 16ug/ml as confirmed by E-test. None of the *Acinetobacters* showed resistance to IMP. Isolates were considered as MBL producers when three of the phenotypic tests were positive. Both DST and CDT (7/8) were superior to Hodge and E-tests (4/8) for detection of MBL production. One IMP resistant isolate was negative by all tests suggesting non-MBL production. None of the IMP resistant isolates was an ESBL producer. In conclusion the majority of our IMP resistant *P. aeruginosa* isolates seemed to be MBL producers. Genetic confirmation and analysis of MBL producers is mandatory for positive isolates screened by phenotypic tests. Among the latter DST and CDT proved to be more rapid and convenient tests for their detection in the clinical laboratory. Testing IMP resistant rather than CAZ resistant isolates could reduce screening work for MBL detection. Colistin may be recommended for treatment of serious infections caused by MDR MBL-positive *P. aeruginosa.*

INTRODUCTION

The rapid and irrepressible increase in antimicrobial resistance of pathogenic bacteria is widely accepted as a major problem that has been observed over the last decade [(1)]. The variety of β-lactamases with wide spectra of substrate specificity and their ability to hydrolyse the majority of β-lactams, consistitute the most important resistance mechanism of GNB. Three major groups of such enzymes are usually distinguished, class C cephalosporinases (AmpC), ESBLs and different types of β-lactamases with carbapenemase activity of which so called MBLs, are of great concern [(2)].

ESBLs are still considered as a threat since they are coded by plasmid and can be easily transmitted between species. ESBL-producing oraganisms are highly effective in inactivating penicillins, most cephalosporins and aztreonam [(3)].

Acquired MBLs are emerging resistance determinants in *P. aeruginosa* and other GNB. These enzymes can hydrolyse all β-lactams, including carbapenems, except monobactams. At least four different types of these enzymes IMP, VIM, SPM and GIM have been identified. The capability of MBLs to dissiminate and spread through bacterial population is facilitated by its being encoded on an intergron-borne mobile gene cassette[(4)] Currently, there are no recommedations available from the CLSI (Clinical and Laboratory Standards Institute) formerly the NCCLS, or elsewhere for the detection of organisms producing MBLs [(5)].

The rapid detection of MBL-producing GNB is necessary to aid infection control and to prevent dissemination. Also infections with MBLs result in higher mortality rates, probably related to less frequent institution of appropriate antimicrobial therapy [(6)] .MBL-activity is

inhibited by chelating agents. Therefore several laboratory methods using metal chelators as EDTA, thiol compounds in combination with β-lactams as IMP or CAZ have been described for the phenotypic detection of MBLs among clinical isolates [7,8] . PCR is currently used for analysis of genetic contex and detection of different types of MBLs [5] .

It has been reported that some MBL-producing GNB are difficult to detect because they are inhibited by low concentrations of IMP, thus they might give false susceptibility result by disk diffusion. So testing all CAZ resistant strains was recommended for detection of MBL production [9,10] . However this was not recommended by other studies [8,11] .

The aim of the current study is to determine the proportion of MBL and ESBL-producers among GNB isolates from clinical MDR pathogens and their antibiotic susceptibility patterns. We also attempted to assess the efficiency of several phenotypic tests for rapid and convenient detection of MBLs among *Pseudomonas* and *Acinetobacter spp*. The efficiency of testing CAZ-resistant versus IMP-resistant pathogens was also compared.

MATERIAL AND METHODS

(I) Bacterial isolates: A total of 70 GNB-MDR consecutive isolates causing infections were collected from hospitalized and ICU patients admitted to Theodor Bilharz Research Institute (TBRI) between January 2004 and June 2005. Isolates exhibited MDR pattern that included resistance to third generation cephalosporins (cefotaxime, ceftazidime and cefuroxime), aminoglycosides (gentamycin and tobramycin) and fluoroquinolones (ofloxacin). The distribution of bacterial isolates among clinical specimens was as follows: urinary tract infections following urosurgical operations (n=22), surgical wound sepsis (n=27), endotracheal tube associated pneumonia (n=12), blood cultures (n=8) and ear discharge (n=1).

(II) Bacterial identification to sepecies level was detected by Vitek-2 automated system(GNI-20and 22) (bioMérieux). Isolated strains were stored at –70^{o}C in a mixture of nutrient broth and glycerol 15% and subcultured once before testing for ESBLs and MBL-production.

(III) Antimicrobial susceptibility to a panel of antimicrobial agents (amikacin, aztreonam, ampicillin, cefaclor, cefipim, cefotaxime, cefoxitine, ceftazidime, cefuroxime, cephalothin, gentamycin, imipenem, meropenem, nalidixic acid, ofloxacin, piperacillin/tazobactam, tobramycin, trimethoprim sulfa, cefpodoxim and colistin) was determined and confirmed by Kirby-Bauer disk diffusion test [12] and by Vitek-2 automated system (GNS-20 and 22).

(IV) Ceftazidime and Imipenem Resistance Determination: In addition to disk diffusion and Vitek-2 susceptibility testing, MICs for CAZ and IMP were evaluated by E test (AB Biodisk, Solna, Sweden) and interpreted according to NCCLS standards [13] .

(V) Detection of Extended-Spectrum β-lactamases (ESBLs): It was performed on all isolates by using oxoid combination test (CDO2) which depends on comparing the inhibition zone given by CAZ (30μg) and CAZ-plus-clavulanate (30μg/10μg) [14] . A difference of ≥ 5mm between the zone of CDO2 and CAZ alone was taken to indicate ESBLs production as advocated by NCCLS methodology [15] . Confirmation of ESBL production by isolates was accomplished by Vitek-2 automated system.

(VI) Phenotypic Detection of Metallo-β-lactamases (MBLs): It was performed on all isolated *Pseudomonas* and *Acinetobacter spp.* Each isolate was tested by 4 methods to detect MBL production. For the first 3 tests, each isolate was suspended in sterile broth and adjusted to a turbidity of 0.5 MacFarland standard then used to swab the inoculate on three Mueller-Hinton agar plate (MH) by a cotton swab according to NCCLS standards. One of the three inoculated MH plates was used for each of the following tests.

1- E test MBL procedure: The IP/IPI E test MBL strips (AB Biodisk, Solna, Sweden) consists of imipenem (IP) (4-256 μg/ml) and IP (1-64μg/ml) plus constant level of EDTA (IPI). After brief drying and E test strip application, plates were incubated for 16-20 hours at 25^{o}C. The MIC end points were read where the inhibition ellipses intersected the strips. A reduction of IP MIC by ≥ 3 two fold dilution in the presence of EDTA (IP/IPI ratio ≥ 8) was

indicative of MBL production. Equally, the presence of a "phantom" zone between two gradient sections or deformation of the IP elipses was also indicative of MBL production [16].

2- Imipenem-EDTA combined disk test (CDT): Two 10μg IMP disks were placed on the dried plate, 10mm apart from edge to edge and 5 ul of 0.5M EDTA solution was then applied to one of the disks to obtain a concentration of 0.750mg/disk. After 24 hours incubation at 37°C, the inhibition zones of IMP and IMP- EDTA disks were compared. For MBL producing organisms, disks with IMP-EDTA increased inhibition zones by 8 to 15 mm (mean, 10.5 mm), while the increase of such zones for MBL-negative isolates was 1 to 5 mm (mean, 3.8 mm) [7].

3- EDTA-disk synergy test (EDST): After plate drying, a 10μg IMP disk and a sterilized blank filter paper disk were placed 10mm apart from edge to edge, and 10 ul of 0.5 M EDTA solution was then applied to the blank disk which resulted in approximately 1.5 mg/disk. After an over night incubation at 37°C, the presence of a synergistic inhibition zone was interpreted as DST positive [17].

4- Modified Hodge test: The surface of MH agar plate was inoculated evenly using a cotton swab with an overnight culture suspension of *E. coli* ATCC 25922, which was adjusted to one-tenth turbidity of 0.5 McFarland standard. After brief drying, an IMP disk (10 μg) was placed at the center of the plate and 10 ul of 50 mM $Zn5o_4$ was added to IMP disk (140μg/disk). Test strains from an overnight culture plates were streaked heavily from the edge of the disk to the periphery of the plate. The presence of a distorted inhibition zone, after overnight incubation at 37°C was interpreted as a positive result for carbapenem hydrolysis screening [8,18].

An IMP-susceptible *P. aeuroginosa* ATCC 27853 was used as negative control for all phenotypic tests.

(VII) Efficiency of screening CAZ resistant versus IMP resistant pathogens for detection of MBLs: All 62 CAZ-resistant/IMP-sensitive GNB isolates recoverd in our study were tested by CDT to detect the presence of MBL-producing strains. The prevalence of MBL-producers among the former group was compared to that detected among CAZ-resistant, IMP-resistant isolates. The disk diffusion susceptibility test results confirmed by Vitek-2 were used to categorize the groups[19].

RESULTS

Of the 70 GNB isolated pathogens, nine species were identified: eight *Enterobacteriacaea spp.* (56 isoltes) and 14 isolates of *P. aeuruginosa* as shown in Table 1.

Prevalence of ESBL Producing Strains and IMP-Resistant Strains:

Among the isolated species, 25 isolates (35.7%) were capable producing ESBLs. *E. coli* and *K. pneumoniae* were the most common ESBL-producing species (66.6% and 61.5% respectively). Meanwhile 11.4% (8/70) of isolates were IMP-resistant and their MIC was 16μg/ml as determined by both E-test and Vitek system. The IMP-resistant isolates were only recovered among *P. aeuruginosa* species with an intraspecies prevalence rate of 57.1 % (Table 1).

Prevalence of MBL-Producing Strains:

MBL-production among the studied GNB isolates was 10% (7out of 70 CAZ-resistant isolates) and 50% (7of14) of *Pseudomonas* isolates were MBL-producers.

Screening for MBLs by Phenotypic Methods:

Phenotypic detection of MBL-producers among CAZ-resistant/intermediate and IMP resistant/sensitive solates of *P. aeruginosa* and *A. baumanii* showed that all tests were negative among IMP-susceptible isolates of both *P. aeruginosa* and *A. baumanii* (Table 2).

The 4 phenotypic tests used in the study showed that both CDT and DST tests were superior to E-test and modified Hodge test. The former 2 tests diagnosed 7 out of 8 isolates as MBL-producers, while the latter 2 tests diagnosed 4 isolates only. One IMP-resistant. *P. aeruginosa* isolate was negative by all 4 tests suggesting non-MBL-production. Isolates were considered potential MBL-producers when at least 3 of the 4 phenotypic tests were positive (Fig. 1).

Antimicrobial Susceptibility Profile of Isolates:

It revealed 32 CAZ resistant strains (MIC ≥ 64 μg/ml) and 38 CAZ intermediate strains (MIC, ≥ 16 μg/ml). The antibiotic susceptibility patterns of the 25 ESBL-producing isolates detected in the study showed that meropenem was the most active with an MIC of 0.25μg/ml. Overall, the rank order of activity in terms of percentage of susceptibility was: meropenem (100%), piperacillin/tazobactam (80%), cefoxitin (98%) amikacin (16%) and ciprofloxacin (4%). All other tested antibiotics including the fourth generation cephalosporine (cefipime) showed absolute resistance.

The antibiotic susceptibility patterns of the 7 imipenem-resistant MBL-producers among *P. aeruginosa* isolates in the study revealed that colistin was the most effective as 87.5% were susceptible with MIC range (0.5-2μg/ml). Meanwhile, a high resistance rate was found for cefatazidime, aztreonam and meropenem (87.5%), to ciprofloxacin 62.5%, to pipracillin/tazobactam 50% and to amikacin and cefipime 25%. While for imipenem-sensitive non-MBL producing strains of *P. aeruginosa*, all isolates (100%) were sensitive to meropenem, 83.3% were sensitive to amikacin and 66% to cefipime (Table 3).

Efficiency of Screening CAZ-Resistant Versus IMP-Resistant Pathogens:

The possible presence of MBL-producing strains among CAZ-resistant but IMP susceptible isolates was examined by screening 62 MDR, GNB pathogens using the CDT. None of them proved to be a potential MBL-producer. Meanwhile the rate of CAZ-resistant and IMP resistant isolates was 11.4% (8/70) and 87.5% (7/8) of them were MBL-producers. Therefore, when screening for MBLs work could be reduced by 77.1% if IMP-resistant rather CAZ-resistant isolates were tested.

Table (1): Prevalence and distribution of ESBLs and IMP-resistance among GNB isolates causing infections

Species	Number of isolates (Interspecies) N (%)	Intraspecies Prevalence of ESBLs N (%)	IMP-R N (%)
E. coli	15 (21.4)	10 (66.6)	--
P. aeruginosa	14 (20)	--	8(57.1)
K. pneumoniae	13 (18.6)	8 (61.5)	--
A. baumanii	7 (10)	--	--
E. cloacae	7 (10)	3 (42.8)	--
M. morganii	5 (7.1)	3 (42.8)	--
C. freundii	5 (7.1)	1 (20)	--
P. vulgaris	2 (2.9)	--	--
Providencia	2 (2.9)	--	--
Total	70(100)	25 (35.7)	8(11.4)

ESBLs: extended spectrum-beta lactamases IMP-R: imipenem-resistant.

Table (2): Phenotypic detection of MBL-producers among CAZ-resistant/intermediate and IMP resistant/sensitive isolates of *Pseudomonas* and *Acinetobacter species*

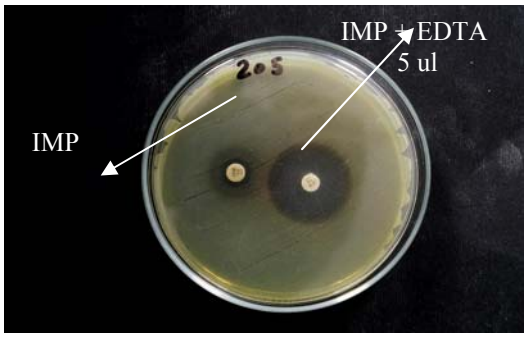
Isolates tested for MBL production(n)	MBL +ve isolates by				No of isolates with the following CAZ/IMP sensitivity			
	Hodge	E test	CDT	EDST	R/R	I/R	I/S	R/S
Pseudomonas								
IMP-sens (6)	**0**	**0**	**0**	**0**	**NA**	**NA**	**6(42.8%)**	**NA**
IMP-res (8)	**4**	**4**	**7**	**7**	**8 (57.1%)**	**NA**	**NA**	**NA**
Acinetobacter								
IMP-sens (7)	**0**	**0**	**0**	**0**	**NA**	**NA**	**4(57.1%)**	**3(42.8%)**
Total (21)	**4**	**4**	**7**	**7**	**8**	**NA**	**10**	**3**

R: resistant, I: intermediate, S: sensitive NA: not applicable

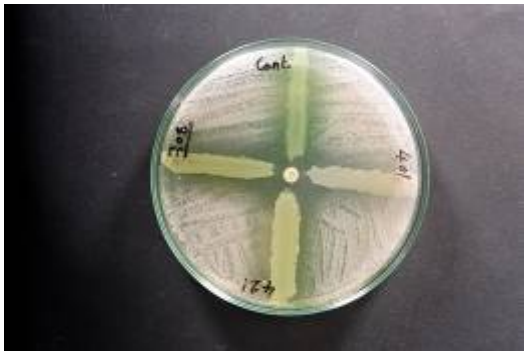
Table (3): Antibiotic susceptibility patterns of phenotypically detected MBL-versus non MBL-producers among *P. aeruginosa* clinical isolates.

Antibiotics	***P. aeruginosa* isolates**					
	Imipenem-resistant MBL – producers (n = 7)			**Imipenem-sensitive non MBL – producers (n = 6)**		
	R %	**I %**	**S %**	**R %**	**I %**	**S %**
Colistin	**12.5**	**0.0**	**87.5**	**0.0**	**0.0**	**100**
Ceftazidine	**87.5**	**12.5**	**0.0**	**0.0**	**100**	**0.0**
Cefipime	**25.0**	**75.0**	**0.0**	**33.0**	**0.0**	**66.6**
Ciprofloxacin	**62.5**	**0.0**	**37.5**	**66.6**	**16.6**	**16.6**
Amikacin	**25.0**	**12.5**	**62.5**	**0.0**	**16.6**	**83.3**
Piperacillin/ tazobactam	**50.0**	**50.0**	**0.0**	**50**	**0.0**	**50.0**
Aztreonam	**87.5**	**12.5**	**0.0**	**0.0**	**66.6**	**33.3**
Meropenem	**87.5**	**12.5**	**0.0**	**0.0**	**0.0**	**100**

Fig. (1): MBL-producers:



(a) CDT: A difference in inhibition zone of 8-15mm (mean 10.5 mm) indicated positive MBL.	(b) EDST: Synergism between the 2 zones of inhibition indicated positive MBL.



(c) E-test (IP/IPI MBL strip): Deformed ellipse around strip indicated positive MBL.	(d) Modified Hodge test: distorted inhibition zone indicated positive MBL.

DISCUSSION

The emergence and dissemination of numerous types of β-lactamases as ESBLs, MBLs and AmpC enzymes among members of GNB population pose a therapeutic challenge. These enzymes collectively can hydrolyze almost all β-lactam drugs which are most frequently used for treatment of serious infections [3] .

The development of simple screening tests that are suitable for routine use in the clinical microbiology laboratory is a critical step toward large-scale monitoring of these enzymes [20] .

Results of the current study revealed that the overall percentage of ESBL-producers among 70 GNB isolates was35.7% by using CDO2 test and confirmed by Vitek automated system. Such alarming result is consistent with previous Egyptian reports from Diab and El-Baz, [21] and Omar et al. [22] (38.8% and 25% respectively).Our result is higher than reports from Japan[23] , Cameron [24] and India [25] (2.8%, 12% and 26.6% respectively).

In the present study, most of ESBL-producers were collected from patients in the ICU and the surgical wards. In these wards isolates are exposed to great antibiotic pressure. Continued use of cephalosporin group appears to be a potential risk factor for emergenence of ESBL-producers [26] . In addition as noted in the present study, the rise of resistance to cefepime (100%) that has been introduced for therapeutic use could be of concern which further limits treatment options. All ESBL-producers were susceptible to meropenem (100%). Currently, carbapenems are regarded as the drug of choice for treatment of infections caused by

ESBL-producers. Unfortunately, extensive carbapenem use has the potential of inducing the emergence of carbapenem-resistant bacterial species particularly in *Pseudomonas spp*.[27] .

Association between MBLs and ESBLs appears to be a rare event in *P. aeruginosa*. In our study, none of the *P. aeruginosa* MBL-producers were ESBLs. However it was reported in one clinical isolate in Argentina. A novel variant of the VIM family in ESBL-producing *P. aeruginosa* has emerged after 8 days of meropenem treatment. Susceptibility analysis showed that the isolate was resistant to all groups of antibiotics except colistin [28] .

MBLs have been identified from clinical isolates worldwide with increasing frequency over the past few years. Of the 70 CAZ resistant GNB isolates in our study, 10% were MBL-producers and 50% of *P. aeruginosa* isolates were MBL-producers. This result was comparable to El-Kholy et [10] (62%) and Pitout et al. [5] (46%) using phenotypic methods among *P. aeruginosa* isolates. Lower prevalence rates (1.9%) were reported from Japan [29] and similar results were reported from Taiwan [30] .

Given the increasing prevalence of MBL-producing GNB in many countries, simple and accurate tests are needed to detect them. Several phenotypic methods are available for the detection of MBL-producing bacteria. All these methods are based on the ability of metal chelatons, such as EDTA and thiol-based compound to inhibit the activity of MBLs. These tests include CDT, EDST, Hodge test, MBL-E test and microdilution test in which EDTA and 1, 10 phenanthrolin with IMP are used [7,8, 16 ,20] .

Most phenotypic methods described for detection of MBLs especially EDST and Hodge test are often difficult to interpret, technically demanding and time consuming, since optimal disk spacing and reincubation of plates are sometimes required to obtain ideal results. Furthermore, 1, 10 phenanthroline is toxic for routine handling [9,17] .

Yan et al. [30] reported excellent sensitivity and specificity for CDT to detect VIM-2 and IMP-1 producing *P. aeruginosa* and *Acinetobacter spp.* MBL-E test is widely available but rather expensive and gives variable results [31] .

In the current study screening for MBL-producers among CAZ-resistant *P. aeruginosa* and *Acinetobacter spp.* revealed that CDT and EDST were superior to Hodge and MBL-E test. Such results were in accordance with Lee et al. [8] , El-Kholy et al. [10] and Pitout et al. [5] who reported that CDT is acceptable method for MBL detection in both IMP-resistant and IMP-sensitive isolates. However, Walsh et al.[16] and El-Kholy et al.[10] revealed higher prevalence rate of MBL-producers by MBL E-test.

In the present study CAZ intermediate/resistant and IMP-sensitive isolates were subjected to screening for detection of MBL-producers. However, MBL-producing isolates were not determined among them. They were only detected among IMP-resistant *P. aeruginosa* strains. This reduced the screening work by 77.1%. Such results were consistent with Lee et al. [17] whose screening work was reduced by 59.6% when testing for MBL production among IMP-resistant rather than CAZ resistant isolates. On the other hand Arakawa et al. [9] and El-Kholy et al. [10] recommended testing CAZ-resistant isolates for MBL production. This discrepancy could be explained by possibility of expression of MBL may be cryptic or may be suppressed in strains demonestrating low level carbapenem resistance [30] . Apparently all our MBL-producers demonestrated high level carbapenem resistance (MIC 16µg/ml). So screening IMP-R strains, that have high level resistance to IMP rather than CAZ-R strains would reduce screening work. When testing for MBLs. Also these strains may have another CAZ-resistance mechanism.

Susceptibility testing of MBL-producing *P. aeruginosa* isolates of the study illustrated that they were more resistant to antimicrobial agents than the non-MBL-producing isolates (87.5, 87.5% and 25% versus 0% for meropenem, ceftazidime and amikacin respectively).

Similar features were found and reported by Pitout et al. [5] . Their MBL-producing *P. aeruginosa* isolates showed absolute resistance to ceftazidime and ciprofloxacin (100%) compared to 30% and 35% resistance rate in the non MBL-producers. A particularly important feature revealed by the study was that colistin was found to be the most active compound (87.5%

susceptible), followed by amikacin (62.5%) and ciprofloxacin (37.5%). Our results were consistent with susceptibility patterns found among MBL-*Pseudomonas* isolates from Brazil where colistin and amikacin were the most efficient antimicrobials (100%, 59%) respectively [32].

As dictated by the susceptibility patterns, it appears that medicine is returning to drugs which have been phased out [33]. Colistin (Polymixin E), the cationic peptide with the potential nephrotoxic effect was successful in treating 14 of 23 critical cases with MDR, MBL-producing *P. aeruginosa* infections [34]. So, until other new efficient agents arise colistin is advocated as the emiprical drug of choice in the setting of MDR-*P.aeruginosa* infections [2].

In an attempt to combat this potential clinical problem, the use of a specific MBL-inhibitor in combination with a β-lactam antibiotic has been approached. A recently discovered series of mercoptocarboxylates have been shown to exhibit both broad spectrum MBL-inhibitory activity and antibacterial synergy with meropenem [35]. Moreover a novel series of tricyclic natural products were extracted from a strain of *Chaetomium furicola* with inhibitory activity against MBLs. These compounds demonstrate the feasability of using MBL-inhibitor/carbapenem combination to tackle this emerging resistance problem in *P. aeruginosa* [36].

On the contrary to the expected, 87.5% of our MBL-positive isolates were resistant to aztreonam in vitro by Vitek system (MIC 32-64μg/ml). This might be probably due to the possible association with an overproducing type of AmpC β-lactamase. Supporting this assumption is that all of these isolates were ESBL non-producers and resistant to cephamycins. AmpC enzymes are cephalospirnases which are poorly inhibited by β-lactamase inhibitors, they confer resistance to third generation cephalosporines, cephamycins, pinicillins and monobactams when produced in large amounts [37]. A similar observation was reported by Lolans et al. [38] who noted that 2 of 6 *P. aeruginosa* MBLs were resistant to aztreonam. Reduced susceptibility to aztreonam could be also due to presence of alternative resistance mechanism.

Resistance to carbapenems in *P. aeruginosa* is often due to impermeability, which arises from loss of the opr D porin or the up-regulation of an active efflux pump system present in the cytoplasmic membrane of the organism [39] or the production of MBLs [40]. This study showed that MBL production is an important cause of IMP resistance among *P. aeruginosa* isolated from our hospital setting as 87.5% of the IMP resistant isolates were MBL positive by phenotypic tests. Pitout et al. [5] reported that 46% of their *P. aeruginosa* clinical isolates were MBL-positive using phenotypic methods (EDST), and 97.1% of the tested isolates were positive for MBL genes using bla_{VIM} and bla_{IMP} genes by duplex PCR amplification. In contrast, a study from Brazil found that 81.1% of their strains were IMP-resistant without MBL production denoting that other resistance mechanisms are involved [41].

In conclusion, our results support the notion that ESBL and MBL production by GNB population is being discovered in our region at an alarming rate. MBL-production among IMP-resistant isolates of *P. aeruginosa* is high and is an infection control issue. Simple phenotypic screening tests as the EDST or the imipenem-EDTA CDT proved to be rapid and convenient tests for their detection in the clinical laboratory. Further studies including more *P. aeruginosa* isolates are required for better evaluation of the problem. Genetic confirmation by PCR and analysis of the genetic context and relatedness of the MBL-producers is mandatory for isolates screened positive by phenotypic tests.

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تحديد الطراز المظهرى لانزيمات الميتالوبيتالكتاميز و البيتالكتاميز واسعة الانتشاربين سلالات العصويات سالبة الجرام المفصولة من حالات اكلينيكية

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يعتبر تحديد انزيمات الميتالوبيتالكتاميز و البيتالكتاميز واسعة الانتشار بين العصويات سالبة الجرام في غاية الاهمية لعلاج المرضى و ضبط انتشار المقاومة.بالرغم من عدم وجود طريقة لتحديد السلالات المنتجة لانزيمات الميتالوبيتالكتاميز و نقص التقارير من مصر: فانه كان من الضرورة اجراء هذا البحث لتحديد نسبة السلالات المفرزة لتلك الانزيم ضمن السلالات العصوية سالبة الجرام بين البكتريا متعددة المقاومة.يهدف البحث إلي تحديد مدي انتشار البكتريا المفرزة لإنزيمات الميتالوبيتا لاكتاميز (MBLs) وإنزيمات البيتا لاكتاميز الواسعة المجال (ESBLs) بين العصويات سالبة-الجرام لكونها أمر حاسم بالنسبة لعلاج المرضي والحد من انتشار مقاومة تلك البكتريا للمضادات الحيوية . ويعد نقص البيانات الموثقة لنسبة انتشارها بمصر من الدواعي الضرورية لتحديد حجم المشكلة .وقد حاولنا تقييم كفاءة عدة اختبارات الطراز الشكلي من أجل تحديد انتشار الميتالوبيتا لكتاميز بطريقة سريعة وملائمة بين سلالات *Acinetobacter, Pseudomonas*. وتم أيضا مقارنة كفاءة اختبار البكتريا المقاومة للسيفتازيديم (CAZ) مقابل البكتريا المقاومة للإميبيتم (IMP). وتم تعيين ٧٠ بكتريا عصوية سالبة الجرام مقاومة أو متوسطة المقاومة للسيفتازيديم (CAZ) واختبار تلك البكتريا للمضـــادات الحيوية بواسطة استخدام نظام الفيتيك الأوتوماتيكي (Vitek-2) . وتم إجراء مسح لإنزيمات البيتا لكتاميز الواســعة المجال (ESBLs) بواســطة اختبـــار CD02 وتم تأكيده باستخدام نظام الفيتيك (Vitek-2)) . وقد تم الكشـــف عن الطـــراز الشـــكلي لإنزيمات الميتالوبيتا لاكتاميز (MBLs) ضمن سلالات *Acinetobacter, Pseudomonas* المعـــزولة بواســـطة الاختبـــارات الآتية Hodge test, EDST, IMP-EDTA, CDT, E-test٠ وقد أسفرت النتائج عن وجود ٢٥ (٣٥,٧%) سلالة مفرزة لإنزيمات البيتا لاكتاميز واسعة النتشار (ESBLs) ومعظمهم ضمن سلالات *K. pneumoniae, E. coli* بينما ٨ (١١. ٤%)من سلالة *P. aeruginosa* كانت مقاومة للايميبينم وكان التركيز الأدنى لإيقاف نمو البكتريا (MIC) بالنسبة لهم هو ١٦ ميكروجرام/مليلتر. لم تظهر أي مقاومة للإميبينم (IMP) ضمن سلالات *Acientobacter*. وقد اعتبرت السلالات مفرزة لإنزيمات الميتالوبيتا لاكتاميز (MBLs) إذا وجد ثلاث اختبارات من الطراز الشكلي موجبة. وقد أظهر كلا الاختبارين CDT, DST (٨/٧) نتائج أعلي من الاختبارين E-test, Hodge test (٤/٨) لتحديد إفراز إنزيمات الميتالوبيتا لاكتاميز (MBLs). لا يوجد أى سلالة مقاومة للإيميبينم (IMP) تفرز إنزيمات البيتا لاكتاميز واسعة المجال (ESBLs) .

ونستخلص من هذا البحث أن معظم السلالات المعزولة المقاومة للإيميبينم (IMP) كانت من سلالة *P. aeruginosa* المفرزة لإنزيمات الميتالوبيتا لكتاميز (MBLs) لذلك كان لزاما إجراء تأكيد وتحليل جيني للسلالات المفرزة لإنزيمات الميتالوبيتا لاكتاميز (MBLs) والتي تم التعرف عليها بواسطة اختبارات الطراز الشكلي. وقد أثبت البحث أن اختباري CDT, DST من أفضل الاختبارات من حيث السرعة والملائمة ويمكن استخدامهما بالمعامل. إن اختبار السلالات المقاومة للإميبينم (IMP) يعتبر مفضلا عن اختبار تلك السلالات المقاومة للسيفتازيديم (CAZ) لأنه سوف يقلل حجم العمل في الكشف عن إنزيمات الميتالوبيتا لكتاميز (MBLs). يمكن التوصية باستعمال الكوليستين (Colistin) لعلاج العدوى الخطيرة المتسببة بواسطة سلالة *P. aeruginosa* المتعددة المقاومة للأدوية والمفرزة لإنزيمات الميتالوبيتا لاكتاميز (MBLs) .