MÆDICA - a Journal of Clinical Medicine

ORIGINAL PAPER

MAEDICA – a Journal of Clinical Medicine
2022; 17(1): 134-142
https://doi.org/10.26574/maedica.2022.17.1.134

Detection of Metallo Beta-Lactamase (MBL) Producing Pseudomonas aeruginosa in a Tertiary Care Hospital, Ghanpur, Medchal, India

A. RADHIKA[a], Jyothi Tadi LAKSHMI[b], Kaliappan ARIYANACHI[c], Varatharajan SAKTHIVADIVEL[d]

[a]Department of Microbiology, Telangana Institute of Medical Sciences, Gachibowli, India

[b]Department of Microbiology, All India Institute of Medical Sciences, Bibinagar, India

[c]Department of Anatomy, All India Institute of Medical Sciences, Bibinagar, India

[d]Department of Medicine, All India Institute of Medical Sciences, Bibinagar, India

ABSTRACT

Introduction: Pseudomonas aeruginosa *is a leading cause of nosocomial infections and is especially prevalent among patients with burn wounds, cystic fibrosis, acute leukemia, organ transplants and intravenous-drug addiction. Acquired metallo-β-lactamases (MBLs) are carbapenemases which require zinc in the active site and are predominantly produced by* P. aeruginosa. *They belong to Ambler's class B and Bush-Jacoby Medeiros Group 3 and hydrolyse virtually all β-lactam agents, including the carbapenems. In India, only blaVIM and NDM-1 have been reported in* P. aeruginosa. *Metallo beta-lactamases have recently emerged as one of the most worrisome resistance mechanisms owing to their capacity to hydrolyse all beta-lactams, including carbapenems.*

Aims and objectives: *The present study aimed to investigate the prevalence and resistance patterns of MBL-producing* P. aeruginosa *in a tertiary care hospital of Ghanpur, Medchal, India, and to compare the effectiveness of two different methods of screening and detecting MBL-producing* P. aeruginosa *in order to formulate a policy of empirical therapy and to take preventive measures in hospital settings.*

Methodology: *In the present study, 60 isolates of* Pseudomonas aeruginosa *were obtained from various clinical specimens, including pus, urine, burns, wound, sputum, pleural fluid, and CSF, which were taken from inpatients and outpatients admitted to MIMS, Ghanpur, India. The study period was from January 2017 to July 2018. The microbial isolates were studied for the detection of the prevalence of MBL production, including their antibiogram.*

Results: *Of the 60* Pseudomonas aeruginosa *isolates, 12 were Imipenem resistant, of which nine were MBL producers. Most isolates (14) were collected in the age group of 21-30 years, followed by that of 31–40 years (13) and 1-10 years (2). Of the total number of samples, 40 strains were isolated*

Address for correspondence:
Dr Lakshmi Jyothi, Additional Professor
Department of Microbiology, All India Institute of Medical Sciences,
Bibinagar Hyderabad Metropolitan Region, Telangana 508126, India
Email: drlakshmijyothitadi@gmail.com

Article received on the the 17th of December 2021 and accepted for publication on the 31st of March 2022

in male subjects, with a male-female ratio of 2:1. Total wound swabs accounted for 40% of the studied specimens, followed by ear swabs (20%) and sputum samples (18.3%). Wound swabs also included most Imipenem-resistant isolates (41.6%). Metallo beta-lactamase producers accounted for 75% of all carbapenem-resistant isolates, using the combined disc method and E-test. By comparison, DDST retrieved 41% of Pseudomonas MBL producers. Isolates were 100% sensitive to Polymyxin-B and showed a 44.4% sensitivity to Piperacillin/Tazobactam, followed by 22.2% for Amikacin and Tobramycin and 11.1% for Ciprofloxacin and Gentamicin.

Conclusion: *The study found a relatively high prevalence of* Pseudomonas *MBL producers (9/60) with 100% Polymyxin susceptibility. Hence, our results warn against an expected high use of Polymyxins in clinical settings. Additionally, the study supports the use of E-tests, CDST and DDST for the screening of* Pseudomonas *MBL producers in regions where PCR detection cannot be performed.*

Keywords: *Pseudomonas aeruginosa*, metallo beta-lactamase (MBL), Imipenem-resistant, combined disk synergy test (CDST), double disk synergy test (DDST).

INTRODUCTION

Pseudomonas aeruginosa is a leading cause of nosocomial infections and is especially prevalent among patients with burn wounds, cystic fibrosis, acute leukaemia, organ transplants and intravenous-drug addiction (1). The most serious infections include malignant external otitis, endophthalmitis, endocarditis, meningitis, pneumonia, and septicaemia (2). P*seudomonas* infection is a cause of concern for treating physicians because of its numerous intrinsic and acquired mechanisms of drug resistance. Although antibiotic resistance in *P. aeruginosa* is caused by multiple mechanisms, the production of carbapenemases is a growing factor leading to resistance (3). Acquired MBLs are carbapenemases which require zinc in the active site and are predominantly produced by *P. aeruginosa*. They belong to Ambler's Class B and Bush-Jacoby Mederios Group 3 and hydrolyse virtually all β-lactam agents, including carbapenems. In India, only blaVIM and NDM-1 have been reported in *P. aeruginosa*.

Metallo beta-lactamases (MBL) have recently emerged as one of the most worrisome resistance mechanisms owing to their capacity to hydrolyse all beta-lactams, including carbapenems (4). Acquired MBL genes are located on integrin structures that reside on mobile genetic elements such as plasmids or transposons, thus enabling widespread dissemination. Currently, the most widely accepted method for the detection and confirmation of MBL production in *P. aeruginosa* is the MBL E-test. Alternative detection methods include the double-disk synergy test (DDST) and combined disk (CD)/disk potentiation test (DPT), with good sensitivity and specificity along with a lower cost. Genotypic methods are reliable and highly accurate, but their accessibility is often limited to reference laboratories. In India, the prevalence of MBL production in *P. aeruginosa* varies from one region to another between 7% and 65% (5).

Aims and objectives

The present study aimed to investigate the prevalence and resistance patterns of MBL-producing *P. aeruginosa* in a tertiary care hospital of Ghanpur, Medchal, India, and to compare the effectiveness of two different methods of screening and detecting MBL-producing *P. aeruginosa* in order to formulate a policy of empirical therapy and to take preventive measures in hospital settings. ❑

MATERIALS AND METHODS

In the present study, 60 isolates of *Pseudomonas aeruginosa* were obtained from various clinical specimens, including pus, urine, burns, wound, sputum, pleural fluid, and CSF, which were taken from inpatients and outpatients admitted to MIMS, Ghanpur, India. The study period was from January 2017 to July 2018. Microbial isolates were studied for the detection of the prevalence of MBL production, including their antibiogram.

Inclusion criteria

1. Isolates from all clinical samples
2. Patients of all ages and both sexes
3. Isolates showing resistance to Imipenem were only tested for the production of MBLs.

Exclusion criteria

1. Isolates sensitive for third generation Cephalosporins were not tested for MBLs.

2. Doubtful isolates were excluded.

All samples were collected under aseptic precautions using standard procedures and processed according to standard guidelines (6, 7).

Direct smear study

Direct smears with Gram stain were screened for the presence of inflammatory cells and type of microbial flora. Gram-stained smears show Gram-negative bacilli along with pus cells.

Specimens were inoculated on blood agar and MacConkey's agar plate.

Brain heart infusion broth was used for the blood culture. The bottle was examined duly for turbidity and subculture was made at regular intervals on to blood agar, MacConkey's agar, and any growth was further processed for identification.

Identification of *Pseudomonas aeruginosa*

A. Culture on blood agar yielded dark-colored flat irregular colonies with β-hemolysis.

B. Non-lactose-fermenting colonies: irregular, flat colonies with bluish-green pigmentation on MacConkey's agar.

C. Colonies have a characteristic fruity odour.

D. Colonies were further identified by several biochemical reactions (Table 1).

TABLE 1. Identification of *Pseudomonas aeruginosa* according to biochemical tests

Test	Result
Oxidative fermentation	Oxidative
Catalase	+
Oxidase	+
Nitrate reduction	+
Indole	-
Biochemical test	Result
Citrate	+
Urease	+
H2S production	-
TSI	Alkaline slant/no change
Arginine hydrolase	Positive
Mannitol fermentation	-
Sucrose fermentation	-

TABLE 2. Antibiotic susceptibility testing in accordance to CLSI

Antibiotic discs	Concentration in µg	Sensitive zone in mm	Intermediate zone in mm	Resistant zone in mm
Cefotaxime (Ce)	30	>23	14-23	<14
Ceftazidime (Ca)	30	>18	14-18	<14
Gentamicin(G)	10	>15	12-15	<12
Amikacin (Ak)	30	>17	14-17	<14
Tobramycin (Tb)	30	>15	12-15	<12
Ciprofloxacin (Cf)	5	>21	15-21	<15
Imipenem (I)	10	>16	13-16	<13
Piperacillin/Tazobactam (PIT)	100/10	>18	-	17
Polymyxin-B (PB)	50	>12	-	11

Antibiotic susceptibility testing

The isolates were subjected to antibiotic susceptibility testing using Kirby Bauer disc diffusion techniques according to the CLSI guidelines. In the present study, susceptibility was tested against several antibiotics procured commercially from Hi-Media Laboratories Ltd, Mumbai. The diameter of the zone was measured and interpreted according to the CLSI guidelines (Table 2).

Methods for detection of MBL production

Imipenem (IMP)-EDTA combined disc test (CDST)

Test organisms were inoculated onto plates with Mueller Hinton agar, as recommended by the CLSI. A 0.5 M EDTA solution was prepared by dissolving 18.61 g in 100 mL of distilled water and adjusting the pH to 8.0 by the usage of NaOH. The mixture was sterilised by autoclaving, two 10 µg Imipenem disks were placed on the plate, and appropriate amounts of 10 µL of EDTA solution were added to one of them to obtain the desired concentration (750 µg). The inhibition zones of the Imipenem and Imipenem-EDTA (Imp-EDTA) disks were compared after 16–18 hours of incubation in air at 35°C. In the combined disc test, if the increase in inhibition zone with the Imipenem-EDTA disc was ≥ 7 mm the Imipenem disc alone was considered MBL-positive.

Impinem-EDTA double-disk synergy test (DDST)

Test organisms were inoculated onto plates with Mueller Hinton agar, as recommended by the CLSI. An Imipenem (10 µg) disc was placed 20 mm center to center from a blank disc containing 10 µL of 0.5 M EDTA (750 µg). Enhance-

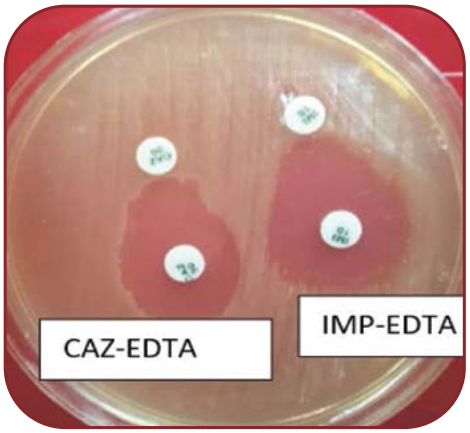

FIGURE 1. Impinem-EDTA double-disk synergy test (CAZ=Ceftazidime, IMP-EDTA= Impinem-EDTA)

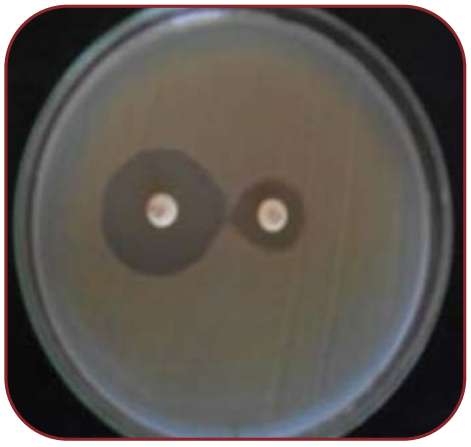

FIGURE 2. MBL E-test

ment of the zone of inhibition in the area between the imipenem and the EDTA disc was >5 mm (Figure 1).

E-test MBL

E-test MBL strips consist of a double-sided seven-dilution range of Imipenem IP (4–256 µg/mL) and IP (1–64 µg/mL) overlaid with a constant 36 gradient EDTA. Individual colonies were picked from overnight agar plates and suspended in 0.85% saline to a turbidity of 0.5 Mc Farmland's. A sterile cotton swab was dipped into the inoculum suspension, and a lawn culture of the inoculum was performed on an MHA plate. Excess moisture was allowed to be absorbed for approximately 15 minutes before the E-test MBL strip was applied. The plates were incubated for 16–18 h at 37°C. The MIC endpoints were read where the inhibition ellipses intersected the strip (Figure 2). A reduction of imipenem MIC=3 two-fold in the presence of EDTA was interpreted as being suggestive of MBL production.

RESULTS

Sixty *Pseudomonas aeruginosa* isolates were obtained from the following clinical samples: pus, urine, wounds, sputum, CSF, ascitic fluid, and pleural fluid.

Of the 60 *Pseudomonas aeruginosa* isolates, 12 were Imipenem resistant, of which nine were MBL producers, and the remaining three non-MBL producers.

The age of the subjects in the study group varied from 1 to 80 years. The maximum number of isolates (14) were found in the age group of 21-30 years, followed by that of 31–40 years (13), while the least number of isolates (2) was from the age group of 1-10 years (Table 3).

Of the total number of samples from all participants (male:female ratio 2:1), 66.7% were from male subjects (Table 4).

Total wound swabs constituted the majority of specimens, accounting for 40%, followed by ear swabs (20%) and sputum samples (18.3%) (Table 5).

Wound swabs constituted the majority of Imipenem-resistant isolates (41.6%), followed by

Age group (years)	Number of cases	Percentage (%)
1-10	2	3.3
11-20	4	6.6
21-30	14	23.3
31-40	13	21.6
41-50	7	11.6
51-60	5	8.3
61-70	7	11.6
71-80	6	10
81 and above	2	3.3
Total	60	100

TABLE 3. Distribution of cases by age group

TABLE 4. Distribution of cases by sex

Participants' sex	No. of cases	Percentage (%)
Male	40	66.7%
Female	20	33.3%
Total	60	100%

TABLE 5. Distribution of various specimens included in the study

Samples	No. of samples	Percentage (%)
Sputum	11	18.3
Ear swabs	12	20
Wound swabs	24	40
Pus	5	8.3
Urine	6	10
Other body fluids	2	3.33
Total	60	100

TABLE 6. Imipenem resistance among various clinical specimens

Specimen	No. of cases	Percentage (%)
Ear swabs	2	16.6
Sputum	3	25
Wound swabs	5	41.6
Urine	1	8.3
Pus	1	8.3
Total	12	100

sputum (25%), ear swabs (16.6%), urine isolates (8.3%) and pus (8.3%) (Table 6).

The incidence of MBL by combined disc method and E-test accounted for 75% and DDST for 41%, and all tests were negative for ATCC strain 27853 of *P. aeruginosa* (Table 7).

Of the 60 *Pseudomonas aeruginosa* isolates, 12 were Imipenem-resistant, of which nine (15%) were MBL producers (Table 8).

In the present study, nine MBLs were isolated from 12 Imipenem-resistant isolates; therefore, the percentage of MBLs in Imipenem-resistant isolates was 75% (Table 9).

Wound swabs showed most MBL-producing isolates, accounting for 44.5%, followed by sputum (22.2%), ear swabs (11.1%), urine (11.1%) and pus (11.1%) (Table 10).

Out of all isolates, 71% were resistant to Cefotaxime and 55% to Ceftazidime. While resistance to Imipenem was noted in 20% of isolates, there was 28% resistance to Piperacillin/Tazobactam and 0% to Polymyxin-B (Table 11).

Tests	Control (ATCC 7853 *P. aeruginosa*)	No. of MBL producers	Percentage positivity (%)
Imp-EDTA CDST	Negative	9	75%
DDST	Negative	5	41%
MBL E-test (MIC test)	Negative	9	75%

TABLE 7. MBL detection – comparison by different methods (N=12)

Total isolates		MBL producers	
		Number	%
P. aeruginosa	60	9	15

TABLE 8. Prevalence of MBL producing *P. aeruginosa* isolates

Organism	Total of Imipenem-resistant isolates	MBL producers		Non-MBL producers	MBL
		Number	%	Number	%
P. aeruginosa	12	9	75	3	25

TABLE 9. MBL and non-MBL producers among Imipenem-resistant isolates

Specimen	No of isolates	MBL production	Percentage (%)
Ear swabs	2	1	11.1%
Sputum	3	2	22.2%
Wound swabs	5	4	44.5%
Urine	1	1	11.1%
Pus	1	1	11.1%
Total	12	9	100%

TABLE 10. Incidence of MBL production among Imipenem-resistant isolates

TABLE 11. Resistance pattern of *P. aeruginosa*

Antibiotic	No. of isolates	Percentage (%)
Cefotaxime	43	**71.6**
Ceftazidime	33	**55**
Gentamicin	20	**33**
Amikacin	**16**	**26**
Tobramycin	**14**	**23**
Ciprofloxacin	**30**	**50**
Imipenem	**12**	**20**
Piperacillin/Tazobactam	**17**	**28**
Polymyxin-B	0	0

TABLE 12. Sensitivity pattern of MBL positive and MBL negative isolates

Antibiotic	MBL positive isolates (n=9)		MBL negative isolates (n=3)	
	Number	%	Number	%
Cefotaxime	0	0	0	0
Ceftazidime	0	0	0	0
Gentamicin	1	11.1	0	0
Amikacin	2	22.2	1	33.3
Tobramycin	2	22.2	1	33.3
Ciprofloxacin	1	11.1	1	33.3
Imipenem	0	0	0	0
Piperacillin/Tazobactam	4	44.4	2	66.6
Polymixin-B	9	100	3	100

MBL producers were 100% resistant to Cefotaxime, Ceftazidime and Imipenem. Isolates were 100% sensitive to Polymyxin-B, 44.4% to Piperacillin+Tazobactam, 22.2% to Amikacin and Tobramycin, and 11.1% to Ciprofloxacin and Gentamicin (Table 12). ❑

DISCUSSION

The first MBL was reported from *Bacillus cereus* in the 1960s, and since then, 18 MBLs have been described in different Gram-negative bacteria. The production of most MBLs is chromosomally encoded and does not pose a serious threat to other bacteria. However, in 1991, the first plasmid-mediated MBL, IMP-1 from *Pseudomonas aeruginosa*, was reported in Japan, while another type of acquired MBL, VIM-1, was first reported in Italy in 1999 (8).

In the present study, the number of *P. aeruginosa* isolates was bigger in the age group of 21-60 years (65%). A similar observation was made by Srinivas *et al* (9) from the Rajiv Gandhi Institute of Medical Sciences, Srikakulam, India, in a study carried on from February 2010 to January 2012, which showed that the isolation of *P. aeruginosa* was more common in the age group of 21-60 years (66.67%). Another similar study conducted by Javiya (10) showed that 61.6% of samples were isolated in the age group of 21-60 years in a tertiary care hospital in Gujarat, India, in 2006.

In the present study, the rate of isolation was more common in men (66.6%) than women (33.3%), with a male to female ratio of 2:1. A similar finding was reported by Rajat Rakesh *et al* (11) from the B. J. Medical College, Ahmedabad, India, in a study performed between April 2009 and April 2010, which showed a male to female ratio of 1.56:1. Also, the study conducted between January 2000–December 2004 by Jamshed Ali *et al* (12) from Hayatabad Medical Complex, Peshawar, Pakistan, found a 1.61:1 sex ratio. In 2006, Viren A. Varaiya (10) from a tertiary care hospital in Gujarat, India, reported a male to female ratio of 2:1.

In the present study, wound swabs constituted 40% of all specimens, followed by sputum (18%) urine (10%) and other body fluids (3%), similarly to the findings reported in Wankhede's study conducted at BJMC, Pune, India, from June 2007 to June 2008 (13), which showed that wound swabs constituted 44.11% of all specimens, followed by urine (25.29%), other body fluids (11.76%) and sputum (14%). This study differs from that conducted by Arora (14) at Adesh Medical College, Bathinda, Punjab, India, from March 2009 to March 2010, in which the maximum number of isolates were from urine (36%), followed by wound swabs (28%), blood (14%), sputum (10%), tracheal aspirate (8%), and other body fluids (4%).

In the present study, the antibiogram of 60 *P. aeruginosa* isolates showed more resistance against Cefotaxime (71.6%), followed by Ceftazidime (55%), similarly to the observation done by Bijayini Behera (15) from AIIMS, New Delhi, India, during 1-30 April 2007, who found a resis-

tance of 78% against Cefotaxime and 67% against Ceftazidime.

This study differs from the observations done by Dwivedi *et al* (16) in an intensive care unit of SGPGIMS, Lucknow, for a period of two years (July 2005–June 2007), who reported a resistance of 90% to Cefotaxime and 85% to Ceftazidime. This study differs from the findings of Zahra Tavajjohi (17), who conducted a study in a tertiary care teaching hospital in Tehran, Iran, in 2010, and reported a resistance of 63% against Cefotaxime and 35% against Ceftazidime.

In the present study we found a 50% resistance against Ciprofloxacin, which is similar to the observations made by Angadi *et al* (18), from June 2010 to December 2010, at Dr. D. Y. Patil Medical College and Research Centre, Pimpri, Pune, India, showing a Ciprofloxacin resistance of 60%. Resistance patterns were also similar to another study conducted by KM Mohan Sundaram (19) during a three-year study from 2008 to 2010, at Vinayak Missions Medical College, Salem, showing a Ciprofloxacin resistance of 62.5%. In the present study, the resistance to Imipenem was 20%. These observations are similar to those of Angadi *et al* (18) and Bashir *et al* (20) from the Sher-I-Kashmir Institute of Medical Sciences, Srinagar, from June 2007 to May 2008, showing an Imipenem resistance of 21.6% and 13.42%, respectively. In the present study, resistance to Piperacillin/Tazobactam was found to be 28%.

A study by Prashanth *et al* (21) at BLD Medical College, Bijapur, India, from November 2008 to September 2010, showed a Piperacillin/Tazobactam resistance of 20.62%, which was similar to our study.

In the present study, the prevalence of MBL in clinical isolates was 15%. Similar observations were made by Bashir (20) at the Sher-I-Kasmir Institute of Medical Sciences, Srinagar, from January 2007 to June 2008, with a reported prevalence of 11.66%. Nagaveni *et al* (22) conducted a study in a tertiary care hospital in Gulbarga from March to October 2008 and reported an MBL prevalence of 20%. Different observations were made by Behera *et al* (23) at AIIMS, New Delhi, India, from April to May 2007, A. P. Zavaschki (24) in Brazil, from September 2004 to June 2005, and A. Manoharan *et al* (25) in a multicentric study from 2005 to 2007, who reported an MBL prevalence of 39.56%, 38.4%, and 42.6%, respectively.

In the present study, the prevalence of MBL in imipenem-resistant isolates was 75%, similarly to studies by Varsha Gupta (26) from the Government Medical College, Chandigarh, during 2007, and Behera (23) from AIIMS, New Delhi, who showed that MBL production in Imipenem-resistant isolates was 69.85% and 64.28%, respectively. These findings differ from those reported by Tanzinah Nasrin (27) at Ibrahim Medical College, Dhaka, whose study carried on from January to June 2009 showed that only 43% of Imipenem-resistant isolates produced MBL.

In the present study, MBL producers were mostly found in wound swabs (44.4%), followed by sputum (18.37%), urine (10%) and other body fluids (3.3%), which was in accordance to the findings of Shanthi *et al* (28) from Sri Ramachandra Medical College, Chennai, who showed that respiratory tract samples contributed to 41.8% of MBL producers, followed by urinary tract (25.5%), wound swab (20%), and blood (12.7%). This study differs from that conducted by Basak *et al* (29) from JNMC, Wardha, between June 2008–December 2009, which showed that among MBL producers, wound swabs accounted for 43.7%, followed by urine (37.5%), sputum (6.2%), endotracheal tube secretions (6.2%) and body fluids (6.2%).

In the present study, all Imipenem-resistant isolates were screened for MBL production using the combined disk synergy test (CDST), double-disk synergy test (DDST) and E-test. MBL production in CDST and E-test was 75% and 41 %, respectively. CDST with Imipenem and EDTA with a cut-off > 7 mm positive and negative results were clearly distinguished. A major disadvantage of DDST is its subjective interpretation.

In this study, CDST was a sensitive method for detection of MBL, which was in line with the findings reported by Behera *et al* (23) from AIIMS, New Delhi, India (CDST 88.8% and DDST 57.14%), P. Pandya *et al* (30) from C.U. Shah Medical College, Surendra Nagar, Gujarat, India (CDST 96.3% and DDST 81.48%), Franklin *et al* (31) from the Microbiology Unit, Melbourne, Australia (CDST 100% and DDST 79%) and Shala Mansouri (32) from Kerman University of Medical Sciences, Iran (CDST 59.8% and DDST 46.8%). This differs from the studies con-

ducted by Picao *et al* (33), Sao Paulo, Brazil (CDST 80% and DDST 82.6%) and Siddabathuni Aruna *et al*, from GSL Medical College and General Hospital, Andhra Pradesh, India (CDST 53% and DDST 57%), which showed that DDST proved to be a more sensitive method than CDST.

Only two of the above studies showed that DDST was slightly more sensitive than CDST, but the majority of studies found CDST to be a more sensitive method for the detection of MBL than DDST. In the present study, the Imipenem-EDTA combined disc test and Imipenem-EDTA MBL E-test were equally effective for MBL detection (75%), which was in accordance with B Behera *et al* (23) from AIIMS, New Delhi, India, who found that both combined disc and E-test were equally sensitive for MBL detection. Among MBL producers, the following percentages of resistance to antibiotics was found in the present study: Cefotaxime (100%), Ceftazidime (100%), followed by Gentamicin (89.9%), Ciprofloxacin (89.9%), Tobramycin (78.8%), Amikacin (78.8%), Piperacillin/Tazobactam (56%) and Polymyxin-B (0%).

Our findings were similar to those reported by Shoba KL (34) from Kasturba Medical College, Manipal, India, in a study performed between February 2007 and January 2008, which showed 100% resistance to Tobramycin and 63% resistance to Piperacillin/Tazobactam, in contrast to a study by P. Pandya *et al*, who showed a resistance of 85.19% to Piperacillin/Tazobactam and 96.3% to Gentamicin.

One limitation of the present study resides in the absence of a PCR analysis for the validation of phenotypical methods. Nevertheless, our study shows a good comparative sensitivity for CDST and MBL E-test, as well as a satisfactory result for DDST as a screening test for MBL production. ❑

CONCLUSION

The present study found a relatively high prevalence of *Pseudomonas* MBL producers (9/60) with 100% Polymyxin susceptibility. Hence, our results warn against an expected high use of polymyxins in clinical settings. Additionally, our study supports the use of E-tests, CDST and DDST for the screening of *Pseudomonas* MBL producers in regions where PCR detection cannot be performed. ❑

Conflicts of interest: none declared.
Financial support: none declared.

References

1. **Bodey GP, Boliver R, Fainstein V, Jadeja L.** Infections caused by Pseudomonas aeruginosa. *CLIN Infect Dis* 1983;5:279-313.
2. **Winn W Jr, Allen S, Janda W, Koneman E, et al (Eds).** *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*, 7th edition, Lippincott and Williams, 2017.
3. **Buchunde S, Mendriatta DK, Deotale V, Narang P.** Comparison of disc and MIC reduction with polymerase chain reaction for detection of Metallo-β-lactamase in Pseudomonas aeruginosa. *IJMM* 2012;30:170-174.
4. **Behera B, Mathur P, Das A, et al.** An evaluation of four different phenotypic techniques for detection of metallo-beta-lactamase producing Pseudomonas aeruginosa. *IJMM* 2012;26:233-237.
5. **Rajput A, et al.** Prevalence of Metallo-beta lactamases producing Pseudomonas aeruginosa. *Indian Journal of Basic and Applied Medical Research* 2012;1:304-308.
6. **Collee V, Digcid JG, Fraser JP, et al.** *Practical Medical Microbiology*, 14th edition (Churchill livingstone Edinburgh), 1996, pp 413-418.
7. **Forbes BA, Sahm DF, Weissfeld A.** *Bailey and Scott's Diagnostic Microbiology.* 12th Edition, Mosby Elsevier, St Louis, Missouri, 2007, p 675.
8. **Butt T, Usman M, Naddem R, et al.** Emergence of metallo-beta-lactamase producing Pseudomonas aeruginosa in Pakistan. *J Pak Med Assoc* 2005;55:302-304.
9. **Srinivas B, Lalitadevi D, Narasingarao B.** A Prospective study of Pseudomonas aeruginosa and its Antibiogram in a teaching hospital of a rural set up. *Journal of Pharmaceutical and Biomedical Sciences* 2012;22:1-5.
10. **Javiya VA, Ghatak SB, Patel KR, Patel JA.** Antibiotic susceptibility patterns of Pseudomonas aeruginosa at a tertiary care hospital in Gujarat, India. *Indian J Pharmacol* 2008;40:230-234.
11. **Rajat Rakesh M, et al.** Antibiotic resistance pattern in Pseudomonas aeruginosa species isolated at a tertiary care hospital, Ahmadabad. *National Journal of Medical Research* 2012;2:156.
12. **Khan JA, et al.** Prevalence and resistance pattern of Pseudomonas aeruginosa against various antibiotics. *Pak J Pharm Sci* 2008;21:311-315.
13. **Arora D, Jindal N, Romit KR.** Emerging Antibiotic resistance in Pseudomonas – A challenge. *Indian Journal of Pharmacy and Pharmaceutical Sciences* 2011;3:82-84.
14. **Wanhede SV, Iyer VS, Bharadwaj RS.** The study of MBL producers from gram-negative isolates in ICU and wards.

Indian Journal of Basic and Applied Medical Research 2011;1:38-46.

15. **Behera B, Das A, Mathur P, Kapil A.** High prevalence of carbapenem resistant Pseudomonas aeruginosa at a tertiary care centre in north India. Are we under reporting? *Indian J Med Res* 2008;128:324-325.
16. **Dwivedi M, et al.** Nosocomial cross transmission of Pseudomonas aeruginosa betweenpatients in a tertiary intensive care unit. *IJPM* 2009;5:509-513.
17. **Tavajjohi Z, Moniri R.** Detection of ESBLs and MBL in Pseudomonas aeruginosa in a tertiary-care teaching hospital. *Iranian Journal of Infectious Diseases* 2011;6:18-23.
18. **Angadi KM, et al.** Detection of antibiotic resistance in pseudomonas aeruginosa isolateswith special reference to Metallo-beta-lactamases from a Tertiary care hospital in Western India. *International Journal of Microbiology Research* 2012;4:295-298.
19. **Mohansoundaram KM.** The antimicrobial resistance pattern in the clinical isolates of Pseudomonas aeruginosa in a tertiary care hospital; 2008-2010 (a 3 year study). *Journal of Clinical and Diagnostic Research* 2011;5:491-494.
20. **Bashir D, et al.** Detection of metallo-beta-lactamase (MBL) producing Pseudomonas aeruginosa at a tertiary care hospital in Kashmir. *African Journal of Microbiology Research* 2011;5:164-172.
21. **Peshattawar PD, Peerapur BV.** ESBL and MBL mediated resistance in pseudomonas aeruginosa: An emerging threat to clinical therapeutics. *JCDR* 2011;5:1552-1554.
22. **Nagaveni S, et al.** Wide spread emergence of MDR Pseudomonas aeruginosa. *Indian J Microbiol* 2010;51:2-7.
23. **Behera B, Mathur P, Das A, et al.** An evaluation of four different phenotypic techniques for detection of metallo-beta-lactamase producing Pseudomonas aeruginosa. *IJMM* 2012;26:233-237.
24. **Zavascki AP, et al.** Higher prevalence of Metallo-β-lactamase mediated resistance challenging antimicrobial therapy against Pseudomonas aeruginosa in a Brazilian teaching hospital. *Epidemiol Infect* 2007;135:343-345.
25. **Manoharan A, Chaterjee S, Mathai D.** Detection and characterization of metallo beta lactamase producing Pseudomonas aeruginosa. *IJMM* 2010;28:241-244.
26. **Gupta V, Sidhu S, Chander J.** Metallo-β-lactamase producing non fermentative gram-negative bacteria: An increasing clinical threat among hospitalizedpatients. *Asian Pac J Trop Med* 2012;5:718-721.
27. **Nasrin T, Jilani MDS, Barai L, Haq A.** Metallo-β-lactamase producing Pseudomonas species in a tertiary care hospital at Dhaka city. *Bangladesh J Med Microbiol* 2010;4:43-45.
28. **Shanthi M, Shekar U.** Multi drug resistant Pseudomonas aeruginosa and Acinetobacter baumanii infections among hospitalized patients: risk factors and outcomes. *Journal of Association of Physicians of India* 2009;57:636-645.
29. **Attal RO, Basak S, Mallick SK, Bose S.** Metallo beta lactamase producing Pseudomonas aeruginosa: An emerging threat to clinicians. *Journal of Clinical and Diagnostic Research* 2010;4:2691-2696.
30. **Pandya NP, et al.** Evaluation of various methods for MBL detection in Gram negative bacilli. *International journal of Biological and Medical Research* 2011;2(3):775-777.
31. **Franklin C, Liolios L, Peleg AY.** Phenotypic detection of Metallo-β-lactamase producing gram negative bacilli in the clinical laboratory. *J Clin Microbiol* 2006;44:3139-3146.
32. **Mansouri S, Nourazi F.** Prevalence of β-Lactamase Production and Antimicrobial Susceptibility of Multidrug Resistant Clinical Isolates of Non-Fermenting Gram Negative Bacteria From Hospitalised patients in Kemran, Iran. *Jundishapur Journal of Microbiology* 2012;5:405-410.
33. **Picao RC, Poirel L, Gales AC, Nordmann P.** Diversity of β- lactamases produced by Ceftazidime resistant Pseudomonas aeruginosa isolates causing blood stream infections in Brazil. *Antimicrobial Agents and Chemotherapy* 2009;53:3908-3913.
34. **Shobha KL, Lenka PR, Sharma MK, et al.** Metallo-beta-lactamase production among Pseudomonas species and Acinetobacter species in coastal Karnataka. *Journal of Clinical and Diagnostic Research* 2009;13:1747-1753.