



**MOLECULAR CHARACTERIZATION OF METALLO BETA LACTAMASE
PRODUCING CLINICAL ISOLATES OF PSEUDOMONAS AERUGINOSA**

Shyamasree Nandy *, Ashish K. Asthana, Molly Madan

Post Graduate Department of Microbiology, Subharti Medical College, Swami Vivekanand Subharti University,
Meerut, India.

*Author for Correspondence: Shyamasree Nandy

Post Graduate Department of Microbiology, Subharti Medical College, Swami Vivekanand Subharti University, Meerut, India.

Article Received on 08/10/2015

Article Revised on 31/10/2015

Article Accepted on 24/11/2015

ABSTRACT

Present study was carried out in the Microbiology Dept of Subharti Medical College, Meerut, UP. 56 non duplicate isolates of *Pseudomonas aeruginosa* resistant to either or both Imipenem and Meropenem were collected. **Material and Methods:** Four phenotypic methods were employed for screening of MBL namely Double disc synergy test (DDST) with Imipenem and 750µgEDTA, Combined Disc test with Imipenem EDTA & Meropenem EDTA, E Test with Imipenem EDTA. PCR was done for molecular characterization of the MBL producers. **Results:** Among 56 isolates 13 isolates were found to be MBL producer. 09 of the isolates were carrying blaVIM and 04 were carrying blaIMP gene. No other gene were found. Maximum number of isolates were obtained in the age group 21 to 30 years. No MBL producer was found in Meropenem sensitive isolates. For screening of metallobeta lactamase Meropenem was found to be a better marker than Imipenem. DDST with Imipenem and Meropenem was found to have same specificity and sensitivity. Etest failed to detect MBL in Imipenem sensitive isolates. All the MBL positive isolates showed resistance towards aminoglycosides. Colistin was 100% sensitive and the next effective drug was Amikacin. In most of the MBL positive isolates Aztreonam was resistant. This can be attributed to the co presence of multiple mechanism of resistance in MBL positive isolates.

KEYWORDS: MBL, *Pseudomonas aeruginosa*, bla VIM, bla IMP.

INTRODUCTION

In the recent years medical world is more focused on the increasing trend of nosocomial infection caused by non lactose fermenter gram negative bacteria.^[1] Among the non fermenters one of the most notorious and most frequently encountered pathogen is *Pseudomonas aeruginosa*. *Pseudomonas* is ubiquitous in nature and renowned for its metabolic diversity. Stover et al had sequenced the complete 6.3 Mbp genome of *P. aeruginosa*. The large genome size & genomic complexity explains the adaptations permitting it to thrive in diverse environment.^[2] It is non fermentative and derives its energy from oxidation rather than fermentation of carbohydrates. It can utilize at least 80 organic compounds but can grow on minimal media with only acetate for carbon and ammonium sulphate for Nitrogen. It does not require any organic growth factors.^[3]

It is also known for its intrinsic resistance to antibiotics and its ability to acquire genes encoding resistance determinants. The various resistance mechanism adopted by *Pseudomonas* are production of beta lactamases and aminoglycoside-modifying enzymes, diminished

expression of outer membrane proteins, mutations in topoisomerases, and up-regulation of efflux. Cumulatively the multiple mechanisms of resistance leads to the development of multiple resistant or even "panresistant" strains.^[4]

Metallobeta lactamases were initially recognised in medically unimportant bacterial species which was chromosomally mediated. Hence much importance was not given to this group of carbapenemases. But the view changed when plasmid mediated transferable MBL were reported from Japan in 1991. During the early nineties it was thought that MBL are a local small scale problem being reported from Japan. But by the end of nineties MBLs were reported from different countries and now it has become a global problem.^[5]

MBL can hydrolyse all the beta lactams except aztreonam. CLSI has not laid any specific guidelines for detection of MBLs though many tests are available for their detection. The most accurate method to detect MBL is PCR. But due to high cost and expertise required it cannot be implemented on regular basis on laboratories.

Among the various phenotypic methods Etest are reported to be very convenient and accurate. However the high cost of E test strips is a hindrance for employing it on regular basis. So a search is on for a cost effective easy to perform test.

In India the rate of MBL production varies from as low as 7.5%^[6] to as high as 69.5%.^[7] Hence this study was undertaken to find out the prevalence and resistant pattern in this geographical area which can help to formulate an antibiotic policy in the hospital.

MATERIAL AND METHOD

The strains of *Pseudomonas aeruginosa* were collected from clinical samples including pus, urine, blood, other body fluid (tracheal aspirate etc) swab (i.e swab from pus, wound, throat swab etc). The MIC of Imipenem & Meropenem were determined by broth dilution method.^[8] The isolates that had an MIC >8 µg/ml were considered as carbapenems resistant (CLSI-2012).^[9] Any isolate of *Pseudomonas aeruginosa* that were either or both resistant to Imipenem or Meropenem were screened for MBL production. All 56 isolates were non duplicate. The strains were stocked in 16% glycerol broth at -20 °C.

Control strains used: *P. aeruginosa* ATCC 27853.

Preparation of EDTA: A 0.5 M EDTA solution was prepared by dissolving 93.05 gm of disodium EDTA. 2H₂O (HiMedia Labs), in 500ml of distilled water (Yong *et al.*, 2002). Ph was adjusted to 8 by adding NaOH. The mixture was sterilized by autoclaving. 10 µl of 0.5M EDTA was added to Imipenem and meropenem disc and Blank disc, dried and stored at -20°C for further use. It was seen that EDTA discs retained its efficacy till 16 weeks. EDTA solution can also be stored at -20°C. But adding EDTA during every test is a cumbersome process. Hence, we had prepared the discs, stored and had used them within 16 weeks.

Imipenem (IMP)-EDTA combined disc test

The IMP-EDTA combined disk test was performed as described by Yong *et al.*^[10] Test organisms was inoculated on to plates with Mueller Hinton agar as recommended by the CLSI-2012.^[9] One 10 µg Imipenem disks (HiMedia) and one Imipenem EDTA disc was placed. Opacity was adjusted to 0.5 McFarland opacity standards. The inhibition zones of the Imipenem and Imipenem-EDTA disks was compared after 16 to 18

hours of incubation in air at 35°C. In the combined disc test, if the increase in inhibition zone with the Imipenem and EDTA disc was ≥ 7 mm than the Imipenem disc alone, it was considered as MBL positive.

Meropenem EDTA combined disc test: The MRP-EDTA combined disk test was performed as described by Varaiya *et al.*^[11] Test organisms was inoculated on to plates with Mueller Hinton agar as recommended by the CLS-2012.^[9] One 10 µg Meropenem disks (HiMedia) and one Meropenem EDTA disc was placed. Opacity was adjusted to 0.5 McFarland opacity standards. The inhibition zones of the Imipenem and Meropenem-EDTA disks was compared after 16 to 18 hours of incubation in air at 35°C. In the combined disc test, if the increase in inhibition zone with the Meropenem and EDTA disc was ≥ 7 mm than the Meropenem disc alone, it was considered as MBL positive.

Imipenem-EDTA double disc synergy test (DDST)

This test was performed as described by Lee *et al.*^[12] Organisms were inoculated on to plates with Mueller Hinton agar as recommended by the CLSI (M100-S22). An imipenem (10 µg) (HiMedia) disc was placed 20 mm centre to centre from another EDTA (750 µg) disc. Opacity was adjusted to 0.5 McFarland opacity standards. Enhancement of the zone of inhibition in the area between Imipenem and the EDTA disc in comparison with the zone of inhibition on the far side of the drug was interpreted as a positive result.

MBL Epsilon meter test

The isolates found to be producing MBL was further tested by MBL E Test strip (HiMedia). The strip is coated with mixture of Imipenem + EDTA on upper half with highest concentration tapering downwards and the lower half is coated with Imipenem in a concentration gradient in reverse direction. When the ratio of the value obtained for Imipenem (IPM) : the value of Imipenem + EDTA (IPM+EDTA) was more than to 8 or If zone was observed on the side coated with Imipenem+EDTA & no zone is observed on the opposite side coated with Imipenem the isolate was considered as MBL positive.

Polymerase Chain Reaction for detection of MBL genes

Amplification: The presence of bla IMP, bla VIM, bla NDM gene was tested in all the 56 test isolates. The primer sequence used was as follows.

Target gene	Primer sequence		Amplicon size	Reference
	Forward	Reverse		
IMP	GTTTATGTTTCATACWTCG	GGTTTAAAYAAAACAACCAC	432	13
VIM	TTTGGTTCGCATATCGCAACG	CCATTTCAGCCAGATCGGCAT	500	13
NDM	GGTGCATGCCCGGTGAAATC	ATGCTGGCCTTGGGGAACG	660	14

Polymerase chain reaction was carried out in 50 µl reaction mixture containing 5 µl template, 1 µl forward primer, 1 µl reverse primer, 25 µl PCR buffer (containing dNTPs, Taq polymerase). Samples were then subjected to

initial denaturation at 95°C for 3 minutes, followed by 30 cycles of 95°C for 30 seconds, 45°C for 1 minute for VIM and 66°C for IMP gene, 72°C for 1 minute and a final extension at 72°C for 10 minutes to complete the

elongation of the PCR intermediate products. For detection of NDM gene amplification was carried out under the following thermal cycling conditions: 10 min at 94°C; 36 cycles of amplification consisting of 30 s at 94°C, 40 s at 52°C, and 50 s at 72°C; and 5 min at 72°C for the final extension. Isolates positive for metallo beta lactamase production was found to be carrying either VIM or IMP. NDM was not detected in any of the isolates.

Gel Electrophoresis: The PCR products were analyzed by electrophoresis in 1.0% agarose gel to detect specific amplified product of 432 bp, 500 bp and 660bp by comparing with standard molecular weight marker of 100 base pair (DNA ladder) . The amplified products of

the study samples were visualized by trans-illuminator, photographed by a digital camera and transferred to computer data for labeling and storage.

Antibiotic susceptibility Testing: Antimicrobial sensitivity was performed on Mueller Hinton agar plates by Kirby-Bauer disk diffusion method according to CLSI guidelines.^[9] Following antibiotic disks (Hi media, Mumbai, India) were used ciprofloxacin-5 µg, levofloxacin 5 µg ,gentamicin-10 µg, amikacin-30 µg, tobramycin-10 µg,netillimycin 30 µg ceftazidime-30 µg, piperacillin100 µg,piperacillin100 µg tazobactam 10 µg,cefepime 30 µg, imipenem-10 µg, meropenem-10 µg,colistin (10µg) & aztreonam-30µg. *P. aeruginosa* ATCC 27853 was used as control.

RESULTS

Table 01:Prevalence of MBL production

	No. of isolate	No. of MBL producer	Percentage
<i>Pseudomonas aeruginosa</i>	56	13	23.21%

Table 02:Comparison of the demographic details of MBL-positive and MBL-negative *P. aeruginosa* isolates(Total n=56)

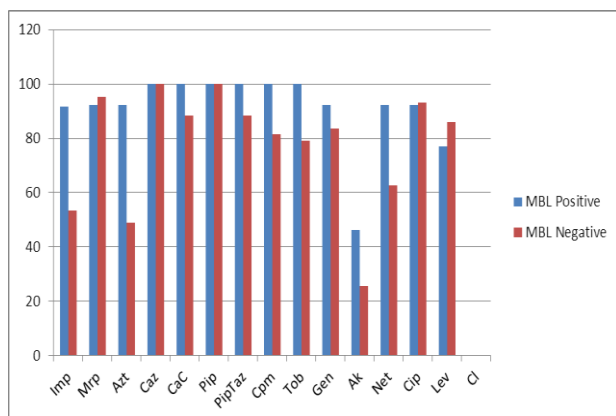
Characteristics	MBL Positive(n=13)	MBL Negative(n=43)	Statistical Analysis
Sex Distribution			
Male	11	34	**The P value =0.764763.
Female	02	09	
Age group distribution			
1-20	0	2	***P-Value =0.832546
21-40	6	24	
41-60	4	11	
61-80	2	6	
** Chisquare test without Yate's correction,two tailed p value,***Chi square test for trend without Yate's correction,two tailed P value.			

Table 03:MIC of Meropenem and Imipenem in MBL positive isolates

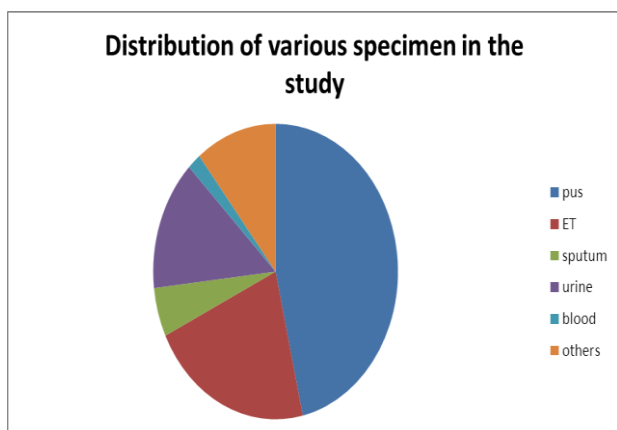
Imipenem	2µg/ml	4µg/ml	8µg/ml	16µg/ml	32µg/ml	64µg/ml	128µg/ml
No of isolates	02	00	5	2	01	03	00
Meropenem	2µg/ml	4µg/ml	8µg/ml	16µg/ml	32µg/ml	64µg/ml	128µg/ml
No of isolates	00	00	02	06	04	01	00

Table 04:Distribution of various genes in the MBL producers(n=13).

Organisms	<i>blaIMP</i>	<i>blaVIM</i>
<i>Pseudomonas aeruginosa</i>	4	9



Comparison of antibiogram of MBL-positive and MBL-negative *P. aeruginosa* isolates(n=56)



Pic:1; IMP and IMP EDTA combined disc test showing increase in zone size of ≥ 7 mm.

DISCUSSION

In India the first report of metallo beta lactamase was published from Bangalore, MS Ramaiah Medical College by Navaneeth et al.^[15] 12% of the isolates included in the study were resistant to both beta lactamase inhibitors and carbapenem. 100% of these isolates were found to be metallo beta lactamase producer.^[15] In India the result of the studies done on metallo beta lactamase producing nonfermentors vary all over the country. The prevalence of metallo beta lactamase producers among carbapenem resistant isolates (Resistant to either or both Imipenem and Meropenem) in the present study was found to be 23.21%. The rate of metallo beta lactamase production in the present study is much lower compared to most of the other studies done in India. However the result is comparable with a study reported by Castanheira et al^[16] and Swami et al.^[17] In this study done by Castanheira et al isolates from 14 of European and Mediterranean countries were included. Overall 20.06% (106 isolates were MBL producer among 529 Carbapenem resistant isolates) *Ps.aeruginosa* isolates were reported to be MBL producer¹⁶. Swami et al from Mumbai reported the rate of MBL production to be 18%.^[17] The difference in prevalence can be attributed to conditions under which they are tested and heterogenous nature of MBLs.

Resistance towards Carbapenems can be conferred by several methods other than production of Metallo beta

lactamase. It has been seen that *P. aeruginosa* can often accumulate different resistance mechanisms leading to carbapenem resistance.^[18] This explains that the isolates included in the study might have shown resistance towards Carbapenems due to enzymes other than metallo beta lactamases or due to loss of porin or due to overproduction of Extended spectrum cephalosporinases or mutation or target alteration.

The isolates included in this study were taken from 45 male patient and 11 female patient. The male to female ratio is 4:1. This is comparable with studies done by Javaiya et al who showed the male female ratio to be 2:1.^[19] The percentage of MBL production in female was found to be 18% which is slightly lower than in male patients (24%).

Among the 56 isolates included 6 were intermediate susceptible, 22 were susceptible and 18 were resistant Imipenem. 2 isolates among Imipenem susceptible isolates were positive for MBL production (09%). This is much lower than the study conducted by Renu et al (20%)^[20] and Zahoor et al (25.21%)^[21] in Imipenem susceptible isolates. Presence of MBL in carbapenem susceptible isolate can be explained by a number of factors. The presence of suppressed MBL gene expression by secondary regulatory systems leads to a silent gene. The plasmid copy number affects MBL gene dosage effect that leads to varied carbapenem hydrolysis.^[22,23]

However only one study demonstrated presence of MBL genes (IMP-16) in Meropenem susceptible isolates.^[23] But in the present study no MBL production was detected in Meropenem susceptible isolates.

The genes detected in the present study are bla IMP and bla VIM. No other gene were detected in the present study. Among 13 MBL producer majority of the isolates had VIM gene (09). Only 04 IMP gene were detected. The study done by Buchunde et al from Mahatma Gandhi Institute of Medical Sciences, Sevagram, Wardha, Maharashtra reported all the MBL producing isolates carrying VIM gene.^[24] From Christian Medical College, Vellore Manoharan et al reported 15 VIM producing isolates among 20 MBL producer.^[25] However all these studies had found single gene responsible for metallo beta lactamase production. A study from Delhi by Niranjana et al had reported presence of multiple genes responsible for MBL production in *Acinetobacter baumannii*.^[26] They also detected NDM-1 in the isolates. However in the present study none of the isolates were found to be NDM producing.

Both MBL positive and negative isolates of *Pseudomonas aeruginosa* showed highest sensitivity towards Colistin followed by Amikacin (sensitivity 10.52%). This is in accordance with the study done by Desmukh et al who also reported the sensitivity of Colistin to be 100% and the next effective drug to be

Amikacin.^[27] Among the other aminoglycosides Netillimycin was the next effective antibiotic (Sensitivity 2.6%) though the sensitivity was quite low. Tobramycin and Gentamycin was 100% resistant in MBL producers whereas it showed 24.4% & 15.55% sensitivity in non MBL producer respectively. Shobha et al also reported 100% resistance towards Tobramycin in MBL producers.^[28] Among the Beta lactam drugs MBL producers were 100% resistant towards Ceftazidime, Ceftazidime Clavulanic acid, Piperacillin, Piperacillin Tazobactam and Cefepime. In the present study resistance towards ceftazidime was 100% in MBL producing isolates. This is in accordance with the study done by Desmukh et al, 2011.^[27] But the study by Ranjan et al reported resistance towards Ceftazidime to be pretty low 29.7%.^[29] Pseudomonas becomes resistant to aminoglycosides by mainly 4 mechanism namely enzyme modification (major), low outer membrane permeability, active efflux and target modification (rarely). they decrease the binding affinity of the modified antibiotics to 30S ribosomal subunit by attaching a phosphate, adenyl or acetyl radical. Accordingly they are divided into three classes: aminoglycoside phosphoryltransferases (APHs), aminoglycoside adenyltransferases (also known as nucleotidyltransferases) (AADs or ANT) and aminoglycoside acetyltransferases (AACs). Generally in Pseudomonas aeruginosa following enzymes are found AAC(6')-II, AAC(3)-I, AAC(3)-II, AAC(6')-I, ANT(2')-I.^[30]

In this study it was seen that all MBL producing Pseudomonas aeruginosa showed aminoglycoside resistance. Highest sensitivity was seen with Amikacin in case of Pseudomonas aeruginosa. Highest resistance was shown towards Tobramycin followed by Gentamicin and Netillimycin. All the MBL producers showed some kind of resistance mechanism towards aminoglycosides. This hints that these organisms carry multiple resistance genes which makes the therapeutic options very limited.

CONCLUSION

The data that was procured in this study suggests that MBL producing organisms almost always carry multiple resistance gene that leaves us with very limited therapeutic options for treatment. These genetic elements which harbors the multiple resistance genes are capable of getting transferred to other organisms posing a serious threat to the nosocomial infections. With strict infection control measures and judicious use of antibiotics the resistance towards carbapenems can be contained.

REFERENCE

1. Gaynes R, Edwards JR. Overview of nosocomial infections caused by gram-negative bacilli. Clin Infect Dis., 2005; 41: 848-54.
2. Stover, C. K., Pham, X. Q., Erwin, A. L., Mizoguchi, S. D., Warrenner, P., Hickey, M. J., ... & Olson, M. V. (2000). Complete genome sequence of

- Pseudomonas aeruginosa PAO1, an opportunistic pathogen. Nature, 406(6799): 959-964.
3. Boone, D. R., Castenholz, R. W., Garrity, G. M., Brenner, D. J., Krieg, N. R., & Staley, J. T. (Eds.). (2005). Bergey's Manual® of Systematic Bacteriology (Vol. 2). Springer Science & Business Media.
4. Bonomo, Robert A., and Dora Szabo. "Mechanisms of multidrug resistance in Acinetobacter species and Pseudomonas aeruginosa." Clinical Infectious Diseases 43. Supplement 2(2006): S49-S56.
5. Bush, K. (2010). Bench-to bedside review: The role of beta-lactamases in antibiotic-resistant Gram-negative infections. Crit Care, 14(3): 224.
6. Gupta, V., Datta, P., & Chander, J. (2006). Prevalence of metallo-β lactamase (MBL) producing Pseudomonas spp. and Acinetobacter spp. in a tertiary care hospital in India. Journal of Infection, 52(5): 311-314.
7. Sharma, M., Yadav, S., & Chaudhary, U. (2010). Metallo-beta-lactamase producing Pseudomonas aeruginosa in neonatal septicemia. Journal of laboratory physicians, 2(1), 14.
8. Bailey W. E. & Scott E. G. (1962). Diagnostic microbiology. Diagnostic microbiology.
9. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 19th informational supplement; Wayne, PA: CLSI; 2012. CLSI document M100-S22.
10. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo-β-lactamases producing clinical isolates of Pseudomonas spp and Acinetobacter spp. J Clin Microbiol., 2002; 40: 3798-801.
11. Varaiya, A., Kulkarni, M., Bhalekar, P., & Dogra, J. (2008). Incidence of metallo-beta-lactamase-producing Pseudomonas aeruginosa in diabetes and cancer patients. Indian Journal of Pathology and Microbiology, 51(2): 200.
12. Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge test and the imipenem-EDTA double disk synergy test for differentiation of metallo-β-lactamases producing clinical isolates of Pseudomonas spp and Acinetobacter spp. J Clin Microbiol., 2003; 41: 4623-9
13. Srinivasan, V. B., Rajamohan, G., Pancholi, P., Stevenson, K., Tadesse, D., Patchanee, P., ... & Gebreyes, W. A. (2009). Genetic relatedness and molecular characterization of multidrug resistant Acinetobacter baumannii isolated in central Ohio, USA. Annals of clinical microbiology and antimicrobials, 8(1): 21.
14. Bonnin, R. A., Naas, T., Poirel, L., & Nordmann, P. (2012). Phenotypic, biochemical, and molecular techniques for detection of metallo-β-lactamase NDM in Acinetobacter baumannii. Journal of clinical microbiology, 50(4): 1419-1421

15. Navaneeth, B. V., Sridaran, D., Sahay, D., & Belwadi, M. R. (2002). A preliminary study on metallo-beta-lactamase producing *Pseudomonas aeruginosa* in hospitalized patients. *The Indian journal of medical research*, 116: 264-267.
16. Castanheira, M., Deshpande, L. M., Costello, A., Davies, T. A., & Jones, R. N. (2014). Epidemiology and carbapenem resistance mechanisms of carbapenem-non-susceptible *Pseudomonas aeruginosa* collected during 2009–11 in 14 European and Mediterranean countries. *Journal of Antimicrobial Chemotherapy*, dku048.
17. Swami, A., Badhuli, S., Desai, P., & Shastri, J. (2014). Incidence of Metallo Beta-Lactamases Producing Non-Fermenters in a Tertiary Care Hospital. *The Indian Practitioner*, 67(4): 219-223.
18. Rodríguez-Martínez, J. M., Poirel, L., & Nordmann, P. (2009). Molecular epidemiology and mechanisms of carbapenem resistance in *Pseudomonas aeruginosa*. *Antimicrobial agents and chemotherapy*, 53(11): 4783-4788.
19. Javiya, V. A., Ghatak, S. B., Patel, K. R., & Patel, J. A. (2008). Antibiotic susceptibility patterns of *Pseudomonas aeruginosa* at a tertiary care hospital in Gujarat, India. *Indian journal of pharmacology*, 40(5): 230.
20. Renu, G., Rajeev, T., & Smita, S. (2010). The existence of metallo beta lactamases in carbapenem susceptible gram negative bacilli: A cause for concern. *J Clin Diagn Res*, 4: 2679-84.
21. Zahoor, D., Fomda, B. A., Thokar, M. A., Bashir, D., Bali, N. K., & Lodhi, S. C. (2014). Carbapenem susceptible metallo beta lactamase producing gram negative bacilli: beware of wolf in sheep dress. *International Journal*, 2(5): 575-583.
22. Peleg, A. Y., Franklin, C., Bell, J. M., & Spelman, D. W. (2005). Dissemination of the metallo-β-lactamase gene blaIMP-4 among gram-negative pathogens in a clinical setting in Australia. *Clinical infectious diseases*, 41(11): 1549-1556.
23. Picão, R. C., Carrara-Marroni, F. E., Gales, A. C., Venâncio, E. J., Xavier, D. E., Tognim, M. C. B., & Pelayo, J. S. (2012). Metallo-β-lactamase-production in meropenem-susceptible *Pseudomonas aeruginosa* isolates: risk for silent spread. *Memórias do Instituto Oswaldo Cruz*, 107(6): 747-751.
24. Buchunde, S., Mendiratta, D. K., Deotale, V., & Narang, P. (2012). Comparison of disc and MIC reduction methods with polymerase chain reaction for the detection of metallo-β-lactamase in *Pseudomonas aeruginosa*. *Indian journal of medical microbiology*, 30(2): 170.
25. Manoharan, A., Chatterjee, S., Mathai, D., & SARI Study Group. (2010). Detection and characterization of metallo beta lactamases producing *Pseudomonas aeruginosa*. *Indian journal of medical microbiology*, 28(3): 241.
26. Niranjana, D. K., Singh, N. P., Manchanda, V., Rai, S., & Kaur, I. R. (2013). Multiple carbapenem hydrolyzing genes in clinical isolates of *Acinetobacter baumannii*. *Indian journal of medical microbiology*, 31(3): 237.
27. Deshmukh, D. G., Damle, A. S., Bajaj, J. K., Bhakre, J. B., & Patwardhan, N. S. (2011). Metallo-β-lactamase-producing clinical isolates from patients of a tertiary care hospital. *Journal of laboratory physicians*, 3(2): 93.
28. Shobha, K. L., Lenka, P. R., Sharma, M. K., Ramachandra, L., & Bairy, I. (2009). Metallo-β-lactamase production among *Pseudomonas* species and *Acinetobacter* species in coastal Karnataka. *Journal of Clinical and Diagnostic Research*, 3(5): 1747-1753.
29. Ranjan, S., Banashankari, G. S., & Babu, P. S. (2014). Comparison of epidemiological and antibiotic susceptibility pattern of metallo-beta-lactamase-positive and metallo-beta-lactamase-negative strains of *pseudomonas aeruginosa*. *Journal of laboratory physicians*, 6(2): 109.
30. Strateva, T., & Yordanov, D. (2009). *Pseudomonas aeruginosa*—a phenomenon of bacterial resistance. *Journal of medical microbiology*, 58(9): 1133-1148.