ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 8.4
IJAR 2023; 9(3): 110-114
www.allresearchjournal.com
Received: 18-01-2023
Accepted: 23-02-2023

Dr. Krishna Kumar Patel
Assistant Professor,
Department of Microbiology
Govt. TCLPG College, Janjgir,
Chhattisgarh, India

Dr. Sarita Patel
Assistant Professor,
Department of Zoology, Govt.
Naveen College, Sakari,
Bilaspur, Chhattisgarh, India

Corresponding Author:
Dr. Krishna Kumar Patel
Assistant Professor,
Department of Microbiology
Govt. TCLPG College, Janjgir,
Chhattisgarh, India

Detection of Metallo Beta-Lactamase (MBL) producing *Pseudomonas aeruginosa*

Dr. Krishna Kumar Patel and Dr. Sarita Patel

Abstract
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 *Pseudomonas 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. The present study aimed to investigate the prevalence and resistance patterns of MBL-producing *Pseudomonas 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 *Pseudomonas aeruginosa* were done in order to formulate a policy of empirical therapy and to take preventive measures in hospital settings.
Of the 160 *Pseudomonas aeruginosa* isolates, 12 were Imipenem resistant; of which nine were MBL producers. Most isolates were collected in the age group of 21-30 years, followed by that of 31–40 years and 1-10 years. Of the total number of samples, 40 strains were isolated 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. The study found a relatively high prevalence of Pseudomonas MBL producers (9/60) with 100% Polymyxin susceptibility. Hence, our preliminary results were against a high use of Polymyxins in clinical settings. Additionally, however this 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)

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 intra-venous-drug addiction. The most serious in- fections include malignant external otitis, endo- phthalmitis, endocarditis, meningitis, pneumonia, and septicemia [1]. *Pseudomonas aeruginosa* infection is a cause of concern for treating physicians because of its numerous intrinsic and acquired mecha- nisms of drug resistance. Although antibiotic re-sistance in *Pseudomonas aeruginosa* is caused by multiple mechanisms, the production of carbapenemases is a growing factor leading to resistance [2]. Acquired MBLs are carbapenemases which require zinc in the active site and are predominantly pro- duced by *Pseudomonas aeruginosa*. They belong to Ambler's Class B and Bush-Jacoby Mederios Group [3] and hydrolyse virtually all Beta-lactam agents, including carbapenems. In India, only blaVIM and NDM-1 have been reported in *Pseudomonas aeruginosa*. Metallo beta-lactamases (MBL) have recently emerged as one of the most worrisome resis- tance mechanisms owing to their capacity to hydrolyse all beta-lactams, including carbapenems [3]. 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 *Pseudomonas aeruginosa* is the MBL E-test.

Alternative detection methods include the double-disk synergy test (DDST) and combined disk (CD) / disk potentiating 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 *Pseudomonas aeruginosa* varies from one region to another between 7% and 65% [4].

Materials and Method

In the present study, 160 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 Chhattisgarh Institute of Medical Sciences, Bilaspur, India. The study period was from January 2022 to December 2022. Microbial isolates were studied for the detection of the prevalence of MBL production, including their antibiogram. Identification of *Pseudomonas aeruginosa*: 1. Culture on blood agar yielded dark-colored flat irregular colonies with Beta-hemolysis. 2. Non-lactose-fermenting colonies: irregular, flat colonies with bluish-green pigmentation on MacConkey's agar. 3. Colonies have a characteristic fruity odor. 4. Colonies were further identified by several biochemical reactions 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 recorded.

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 sterilized by autoclaving, two 10 microgram, Imipenem disks were placed on the plate, and appropriate amounts of 10 microliter of EDTA solution were added to one of them to obtain the desired concentration (750 microgram). 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 microgram) disc was placed 20 mm center to center from a blank disc containing 10 microliter of 0.5 M EDTA (750 microgram). Enhancing the presence of EDTA was interpreted as being suggestive of MBL production.

Results

One sixty *Pseudomonas aeruginosa* isolates were obtained from the following clinical samples: pus, urine, wounds, sputum, CSF, Blood, ET tubes, ascetic fluid, and pleural fluid. Of the 160 *Pseudomonas aeruginosa* isolates, 12 were Imipenem resistant, of which nine were MBL producers, and the remaining three non-MBL producers.

Total wound swabs constituted the majority of specimens, accounting for 40%, followed by ear swabs (20%) and sputum samples (18.3%). Wound swabs constituted the majority of Imipenem resistant isolates (41.6%), followed by sputum (25%), ear swabs (16.6%), urine isolates (8.3%) and pus (8.3%).

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 *Pseudomonas aeruginosa* Of the 160 *Pseudomonas aeruginosa* isolates, 12 were Imipenem-resistant, of which nine (15%) were MBL producers. Nine MBLs were isolated from 12 Imipenem-resistant isolates in this present study; therefore, the percentage of MBLs in Imipenem-resistant isolates was 75%. Pus showed most MBL producing isolates, accounting for 44.5%, followed by sputum (22.2%), ET (11.1%), urine (11.1%) and Blood (11.1%). Out of all isolates, 71% were resistant to Cefotaxime and 55% to Ceftazidime. While resis tance to Imipenem was noted in 20% of isolates, there was 28% resistance to Piperacillin/Tazobac- tam and 0% to Polymyxin-B. 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 Tobramyc in, and 11.1% to Ciprofloxacin and Gentamicin.

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 [5].

In this study we found the number of *Pseudomonas aeruginosa* isolates was bigger in the age group of 21-60 years (65%). Another similar study conducted by Javiya 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 [6].

In 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 [7]. 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 [7].

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 *Pseudomonas 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 resistance 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 Ceftazi dime

[8]. This study differs from the findings of Zahra Tavajjohi, 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 [9]. In the present study, the prevalence of MBL in clinical isolates was 15%. Similar observations were made by Bashir at the Sher-I-Kasmir Institute of Medical Sciences, Srinagar, from January 2007 to June 2008, with a reported prevalence of 11.66% [10]. Nagaveni, *et al*. 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*. at AIIMS, New Delhi, India, from April to May 2007, A. P. Zavaschki in Brazil, from September 2004 to June 2005, and A. Manoharan, *et al*. 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 from the Government Medical College, Chandigarh, during 2007, and Behera from AIIMS, New Delhi, who showed that MBL production in Imipenem-resistant isolates was 69.85% and 64.28%, respectively [11-17].

These findings differ from those reported by Tanzinah Nasrin at Ibrahim Medical College, Dhaka, whose study carried on from Ja- nuary 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* 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* 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*. from AIIMS, New Delhi, India (CDST 88.8% and DDST 57.14%), P. Pandya, *et al*. from C.U. Shah Medical College, Surendra Nagar, Gujarat, India (CDST 96.3% and DDST 81.48%), Franklin *et al* from the Microbiology Unit, Melbourne, Australia (CDST 100% and DDST 79%) and Shala Mansouri from Kerman University of Medical Sciences, Iran (CDST 59.8% and DDST 46.8%). This differs from the studies conducted by Picao, *et al*., 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 [18-23].

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*. 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%) [24].

Our findings supports the study conducted by Shoba KL 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. The Only limitation of the present study is absence of PCR analysis for the validation of phenotypical methods. 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. The present study found a relatively high prevalence of Pseudomonas MBL producers (9/60) with 100% Polymyxin susceptibility. Hence, our results were against a high use of polymyxins in clinical settings. Additionally, our study also, supports the use of E-tests, CDST and DDST for the screening of Pseudomonas MBL producers in regions where PCR detection cannot be performed.

Table 1: Distribution of various specimens included in the study

Samples	No. of Samples	%
Pus	60	37.5
Sputum	40	25
ET Tube	39	24.37
Urine	9	5.62
Blood	7	4.37
Body Fluid	5	3.12
Total	160	

Table 2: Resistance pattern of *P. aeruginosa*

Antibiotics	No of isolates	%
Cefotaxime	43	72
Ceftaidime	33	55
Gentamicin	20	33
Amikacin	16	26
Tobramycin	14	23
Ciprofloxacin	30	50
Imipenem	12	20
Pipracilin/Tazobactum	17	28
Polymycin -B	0	0

Table 3: Sensitivity pattern of MBL positive and MBL negative isolates

Antibiotic	MBL positive isolates		MBL negative isolates	
	Number	%	Number	%
Cefotaxime	0	0	0	0
Ceftaidime	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
Pipracilin/Tazobactum	4	44.4	2	66.6
Polymycin -B	9	100	3	100

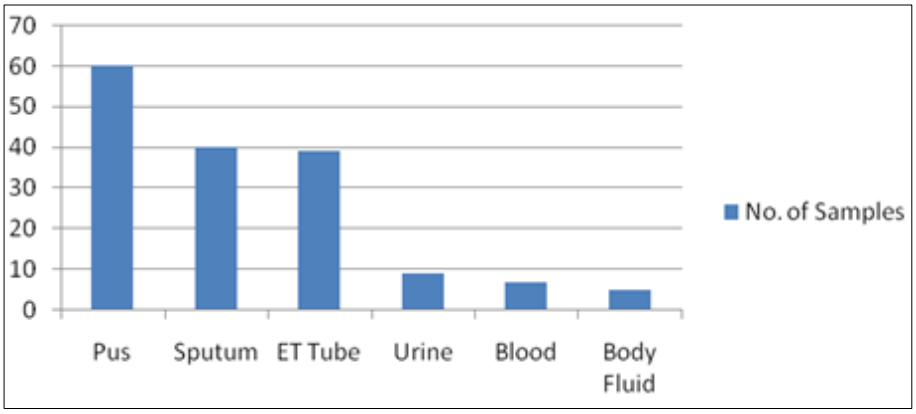

Fig 1: Distribution of various specimens included in the study

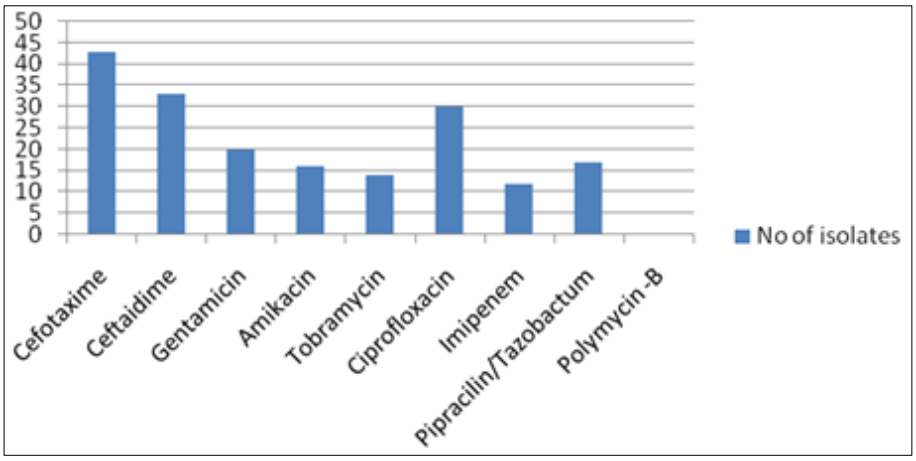

Fig 2: Resistance pattern of *P. aeruginosa*

References

1. 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; c2017.
2. 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.
3. 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.
4. Rajput A, *et al*. Prevalence of Metallo-beta lactamases producing *Pseudomonas aeruginosa*. Indian Journal of Basic and Applied Medical Research. 2012;1:304-308.
5. 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.
6. 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.
7. Dwivedi M, *et al*. Nosocomial cross transmission of *Pseudomonas aeruginosa* betweenpatients in a tertiary intensive care unit. IJPM. 2009;5:509-513.
8. 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.
9. 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.

10. 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.
11. 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.
12. Peshattawar PD, Peerapur BV. ESBL and MBL mediated resistance in *Pseudomonas aeruginosa*: An emerging threat to clinical therapeutics. JCDR. 2011;5:1552-1554.
13. Nagaveni S, *et al*. Wide spread emergence of MDR *Pseudomonas aeruginosa*. Indian J Microbiol. 2010;51:2-7.
14. 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.
15. 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.
16. Manoharan A, Chaterjee S, Mathai D. Detection and characterization of metallo beta lactamase producing *Pseudomonas aeruginosa*. IJMM. 2010;28:241-244.
17. Gupta V, Sidhu S, Chander J. Metallo-β-lactamase producing non fermentative gram-negative bacteria: An increasing clinical threat among hospitalized patients. Asian Pac J Trop Med. 2012;5:718-721.
18. 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.
19. 19 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.
20. 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.
21. 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.
22. 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.
23. 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.
24. 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.