Original Article

An Approach of Phenotypic Detection of Metallo-Beta Lactamase Producing *Pseudomonas aeruginosa*: A Study in Madhya Pradesh

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Abstract

Introduction: Antibiotic resistance in the last decade has recorded drastic changes in an increasing manner in nonfermenting bacterial groups of gram-negative bacilli including *Ps. aeruginosa* is a potent bacterial isolate or opportunistic nosocomial human pathogen. In recent decades, with improvements in antimicrobial therapy and diagnostic procedures have emerged multidrug resistant with beta lactamase to β-lactam antibiotics in presence of carbapenemases group of enzymes as metallo β-lactamases (MBL) are a major cause of concern. Due to its transferable character, it can hydrolyze almost all antibiotics. So, the study has conducted the detection of MBL isolates in hospital. **Materials and Methods:** In the study, 134 nonrepetitive clinical isolates of *Pseudomonas spp.* from the In Patient Department (IPD) patients attended to hospital from different clinical and isolates were confirmed in the department of microbiology and tested by the Kirby-Bauer disc diffusion method, which were screened for MBL production by disc synergy test as double-disc synergy test (DDST), combined-disc synergy test, and Modified Hodge test (MHT). The test was observed by a zone of inhibition of antibiotic with EDTA discs was ≥7 mm antibiotic disc alone as positive. MHT by the presence of a 'cloverleaf shaped' zone of inhibition was considered positive. Data were statistically analyzed and generated the graphs, whereas categorical variables (age and gender) were described in a descriptive way. Prevalence and percentage were used to establish an association of risk factors with carbapenem resistance strains. **Result:** A total of 134 *Ps. aeruginosa* was obtained from various clinical samples as 56 were found maximum in pus. Thirty nine bacterial isolates were found carbapenem resistant and in which 24 males (61.5%) were higher than 15 females (38.5%) in the 41-50 years age group. Carbapenem-resistant strains screened were for MBL production and found maximum by MHT test showed positive with meropenem (76.92%) and imipenem (71.79%). Isolates were found maximum in inpatients from surgical ward 45 (33.58%). Also, carbapenem-resistant isolates were found maximum from surgery (18). Four patients developed surgical site infections, had ulcerative lesions viz. traumatic, nondiabetic ulcer and from intensive care unit, 2 patients clinically identified as ventilated associated pneumonia (with COPD), 1 was immunocompromised with respiratory failure and COPD died in study. **Discussion:** Carbapenem resistant *Ps. aeruginosa* was found especially among critically ill patients. Isolates were found predominantly

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among males in the 41-50 years age group of patients. All 39 isolates were found resistant to meropenem, 32 (82.05%) bacterial isolates were resistant to both meropenem and imipenem where 7 (17.95%) isolates were found resistant to meropenem but sensitive to imipenem. MHT test was used to detect carbapenems activity but it does not give the confirmation of metal dependence of the carbapenems none of the phenotypic tests were optimal due to lower sensitivity or specificity. DDST has less channel of subjective variation, but combined-disc synergy test interprets more. The test between meropenem and meropenem with EDTA disc enhanced by diffusion method demonstrates the better synergy test (also for imipenem) and detection of carbapenem-resistant are not statistically significant [calculated *P* value is. 12 (*P* value >.05)]. The Minimum Inhibitory concentration (MIC) antibiotic susceptibility testing by E-test for imipenem and meropenem defines the use for the future. The surgical department found the highest prevalence may be due to the patient being treated by another hospital and was on third-line medication of cephalosporins. This may be due to higher comorbidity, invasive procedures, nonhealing ulcers after surgery, longer stay in the hospital, and excessive use of broad-spectrum antibiotics. The study was observed in economically weaker patients; seasonal trends of patient's admission and due to rural area tertiary healthcare centers awaiting improvement of the facility which may associate admission avoidance by the patient. **Conclusion:** MBL production was identified as the resistant mechanism of resistance carbapenems group of drugs including another group of antibiotics; prevalence was found similar to other studies but the resistance to another group of antibiotics was found lower. Also, disc diffusion method was reliable for screening with a correlation of DDST or MHT. Our study was focused to improve hospital infection control by the prevalence of organisms and their related risk factors, which may help in care and treatment of patients after admission. Evaluation still needs to conclude a positive association as per our study. E-test–based testing and molecular detection were not done in all isolates.

Keywords: *Carbapenem resistant, carbapenemase, disc synergy test, Gram-negative Bacilli, imipenem, Kirby-Bauer disc diffusion method, meropenem, metallo beta-lactamases, modified Hodge test, Pseudomonas aeruginosa*

Introduction

In the last few decades, resistance to antibiotics has recorded drastic changes with increasing manners. Especially the microorganisms of nonfermenting bacterial groups.[1] Among nonfermenting gram-negative Bacilli, *Ps. aeruginosa* is a potent bacterial isolate or opportunistic nosocomial human pathogen.[2] In recent decades, with improvements in antimicrobial therapy and diagnostic procedures, *Ps. aeruginosa* has emerged as multidrug-resistant (multiple antimicrobial agents).[3,4] Majority of *Ps. aeruginosa* bacterial isolates were identified with varying degrees of beta-lactamase–mediated resistance to most of the beta-lactam antibiotics.[5] Among the broad-spectrum activity of beta-lactamases, the carbapenemases group of enzymes especially metallo beta-lactamases (MBL) are a major cause of concern because of hydrolyzing ability of these enzymes against beta-lactam antibiotics including carbapenem group of antibiotics, which was considered a reserve group of drugs for the treatment of extended spectrum of beta-lactamases producing bacterial isolates.[6] MBL group of carbapenemases, due to the transferable character, it can hydrolyze almost all antibiotics. So, the study has conducted the detection of MBL isolates among *Ps. aeruginosa* isolated in clinical specimens received from clinical departments of tertiary care hospital.

Material and Methods

In the study, 134 nonrepetitive clinical isolates of *Pseudomonas spp* from the patients who attended IPD of the hospital of Amaltas Institute of Medical Sciences, Dewas were isolated in specimens received from different clinical departments. The study used the strains which were isolated from clinical specimens and stored at cold temperatures and checked the prevalence of isolates as carbapenem resistant. The institutional ethical committee was approved for study (AIMS/SRC/2019/M-19/09 dated on 24/09/2019) and isolates were tested in the department of microbiology by Kirby-Bauer disc diffusion method as per Clinical Laboratory Standard Institute (CLSI) guidelines.[7] Isolates were identified as resistant to meropenem and imipenem group of carbapenems antibiotics including another group of antibiotics, which were screened for MBL production by double-disc synergy test,[8,9] combined-disc synergy test,[10] and modified Hodge test (MHT).[11] For the screening test isolates, inoculums were prepared with emulsifying 5-6 colonies of isolates in peptone water to 0.5 McFarland opacity standard. The inoculum was spread on Mueller-Hinton Agar and placed the disc 10 mcg of meropenem and imipenem disc, 10 uL of EDTA [Prepared Ref solution added to sterile blank filter paper Disc (6 mm in diameter, Whatman filter paper no. 2)] [Figure 1].

In the double disc synergy test (DDST- Yong D 2002), two discs used, one without EDTA solution

meropenem disc (10 mcg) and one containing 10 µl of 0.5 M (750 mcg) anhydrous EDTA, were placed 20 mm center to center from a blank disc containing 10 µL of 0.5 M (750 mcg) anhydrous EDTA placed and inoculated bacterial growth on Muller-Hinton Agar after comparison with 0.5 McFarland standard. Then plates were inoculated for 16-18 hours at 37°C. Same procedure was done for imipenem disc (10 mcg) (Hi Media, Mumbai, India).

The test was observed by enhancement of the zone of inhibition between the meropenem with and without EDTA discs and the same procedure was observed for imipenem also. It was interpreted as positive by enhancement of the zone of inhibition between two discs with and without EDTA discs and negative by no enhancement of the zone of inhibition between two discs with and without EDTA discs [Figure 2].

In the combined disc synergy test (CDST, Lee K 2003), two discs used separately, in which one meropenem disc was placed on a Mueller-Hinton Agar plate, one without EDTA solution, and one meropenem disc (10 mcg) with 10 µl of 0.5 M (750 mcg) anhydrous EDTA was placed and inoculated bacterial growth on Muller-Hinton Agar after comparison with 0.5 McFarland standard. Then plates were inoculated for 16-18 hours at 37°C. This test is used for both types of carbapenem disc, 10 mcg meropenem and imipenem disc (Hi Media, Mumbai, India).

The test was observed by a zone of inhibition of meropenem-EDTA discs was ≥7 mm, more than that of meropenem disc alone and the same procedure was observed for imipenem also. Its interpretation was done positively by a zone of inhibition of meropenem-EDTA discs was ≥7 mm, more than that of meropenem disc alone and the same procedure was observed for imipenem also and negative by a zone of inhibition of meropenem-EDTA discs was ≤7 mm, less than that of meropenem disc alone and same procedure observed for imipenem also [Figure 2].

MHT (Lee K 2001, Ramana KV *et al*.[12]): This test identified carbapenem resistant gram-negative Bacilli to detect carbapenemase enzyme production. The carbapenem (Imipenem or Meropenem)–resistant strains were subjected to the MHT for the detection of carbapenemases. An overnight culture suspension of *Escherichia coli* ATCC 25922 adjusted to 0.5 McFarland standard was inoculated using a sterile cotton swab on the surface of Mueller-Hinton agar. After 15 minutes of drying, a 10-mcg Imipenem disc (Hi-Media, Mumbai, India) was placed at the center of the plate and the test strain was streaked from the edge of the disc to the periphery of the plate in four different directions. The plate was incubated overnight at 37ºC. Then interpreted by the presence of a 'cloverleaf-shaped' zone of inhibition due to carbapenemase production by the test strain that was considered as positive with meropenem and imipenem and negative by the absence of 'cloverleaf-shaped' zone of inhibition due to the absence of carbapenemase production by the test strain [Figure 3].

The study selected stored the bacterial isolates which were identified for carbapenem drug resistance and stored at cold temperatures and no approval was required by the institutional ethics committee.

In the study, data obtained were coded and entered into Microsoft word and Microsoft excel to generate the graphs, whereas statistical analysis of categorical variables (age and gender) was described in a descriptive way by percentages and frequencies of various characteristics. Prevalence and percentage were used to

Figure 1: Antibiotic susceptibility testing showing resistant to meropenem and imipenem by kirby baur disc diffusion method Mueller Hinton Agar

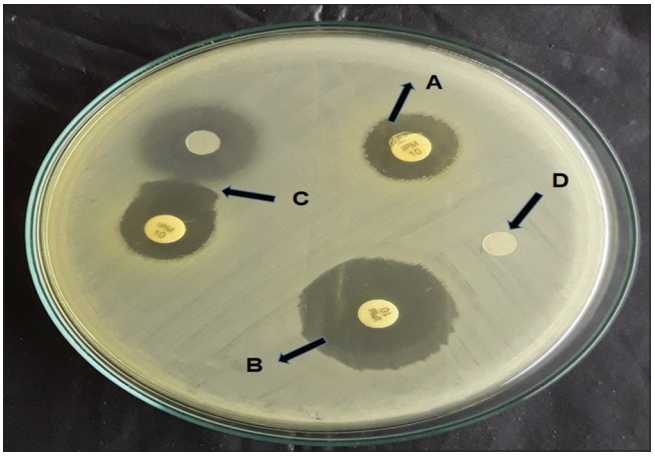

Figure 2: Showing EDTA Disc synergy test for Carbapenem Resistant strains. (A) Antibiotic resistant. (B) Showing combined disc synergy test (Antibiotic incorporated EDTA). (C) Showing double disc synergy test (one antibiotic disc and other disc with EDTA). (D) Showing blank disc

establish an association of risk factors with carbapenem resistance strains.

Result

A total of 134 consecutive nonrepetitive isolates of *Ps. aeruginosa* obtained from various clinical samples were included in the study; 56 were found maximum in pus followed by 39 urine, 18 sputum, 12 blood, 4 endotracheal tubes, 4 aural swabs, and 1 synovial fluid [Figure 4], which were shown with sensitive and resistant strains [Figure 5]. The number of patients with different age groups was found with *Ps. aeruginosa*. The patients with *Ps. aeruginosa* infection were found in 41-50 years age group [Figure 6] among the inpatients and the highest number were from surgical ward 45 (33.58%), followed by orthopedic 31 (23.13%) and the least from pediatric 2 (1.49%).

In the study, isolates were found carbapenem-sensitive 95 (71%) and carbapenem-resistant 39 (29%) [Figure 7]. In the antibiotic susceptibility, all carbapenem-resistant strains were found sensitive to colistin and polymyxin B [Figure 8]. Thirty nine bacterial isolates were found carbapenem resistant, of which isolates were found from males 24 (61.5%) and from females 15 (38.5%). These were screened for MBL production.

Antimicrobial susceptibility testing of *Ps. aeruginosa* isolates were identified in 39 carbapenem-resistant isolates found from surgery (45.2%) followed by intensive care unit (ICU) (28.2%). Isolates were identified from patients of the surgery department, 4 patients documented as surgical site infections, had ulcerative lesions viz. traumatic, and nondiabetic ulcer.

Of 39 carbapenem resistant *Ps. aeruginosa* isolates, 32 isolates were found resistant to both imipenem and meropenem and 7 *Ps. aeruginosa* bacterial strains were found resistant to meropenem only.

Of total 39 isolates of *Ps. aeruginosa* screened for MBL production with meropenem and imipenem disc by diffusion test as EDTA double-disc synergy test EDTA, CDST, and MHT. Of 39 bacterial strains were showed positive by EDTA-DDST meropenem (69.23%), imipenem (64.1%), and by EDTA-CDST meropenem (69.23%) and imipenem (66.67%). By MHT test showed positive with meropenem (76.92%) and imipenem (71.79%). Bacterial strains resistant to three or more antibiotic groups are considered multidrug-resistant strains.

The study found that in the patients, who were resistant to meropenem and imipenem and admitted to ICU, 2 patients clinically identified as ventilated-associated

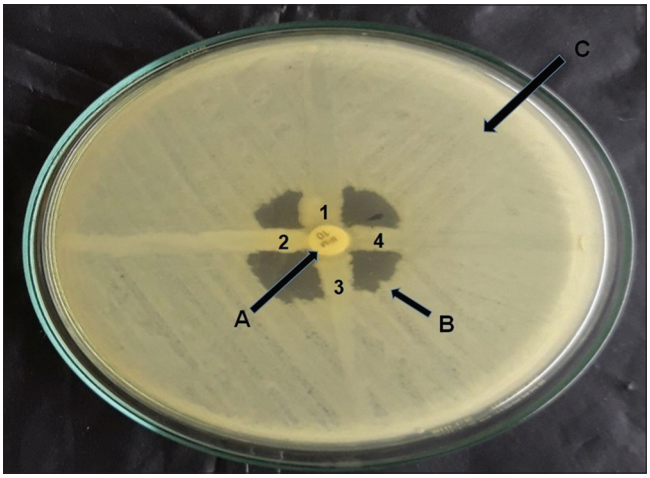

Figure 3: Showing Modified Hodge Test. A. Carbapenem disc. B. The presence of a 'cloverleaf shaped' zone of inhibition due to carbapenemase production by the test strain was considered as Positive. C. Control Strain and Number 1 to 4 – Bacterial Test strain

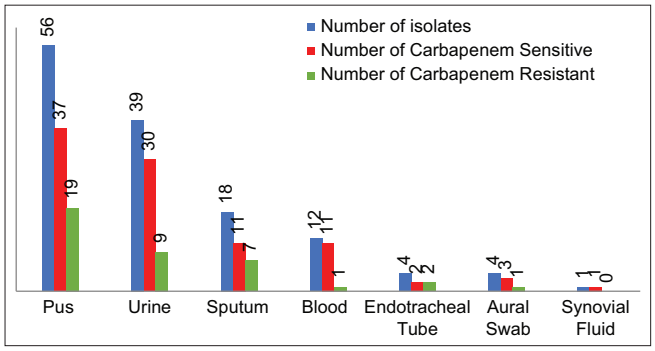

Figure 5: Distribution of *Pseudomonas aeruginosa* in clinical specimens

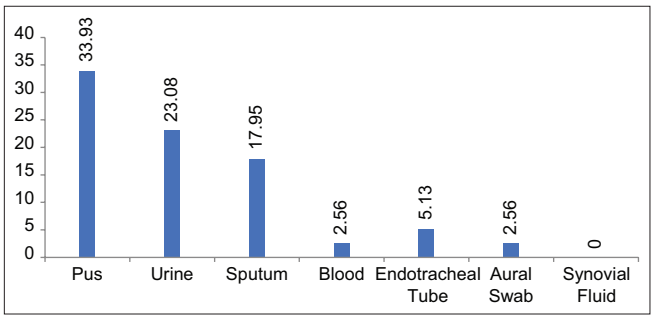

Figure 4: Percentage of Carbapenem Resistant *Pseudomonas aeruginosa* among clinical specimens

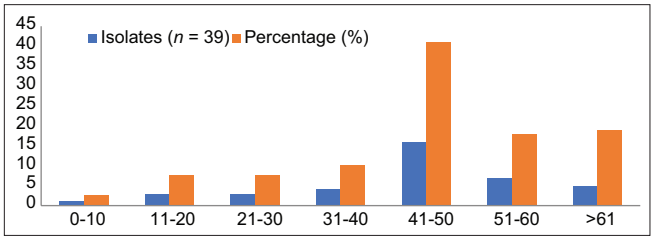

Figure 6: Percentage of Carbapenem resistant *Ps. aeruginosa* distribution in patient of different age group

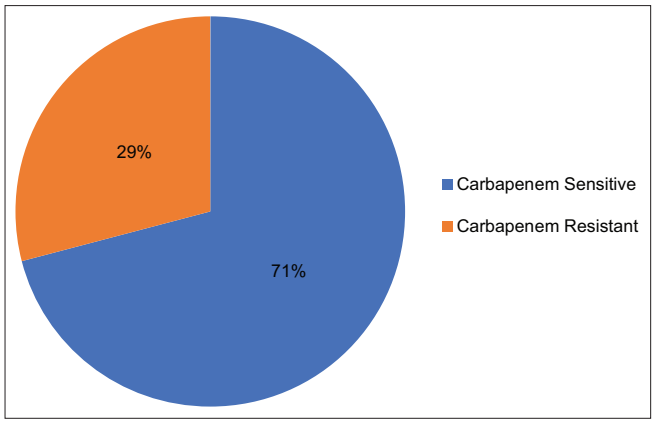

Figure 7: Percentage of antibiotic susceptibility of *Pseudomonas aeruginosa*

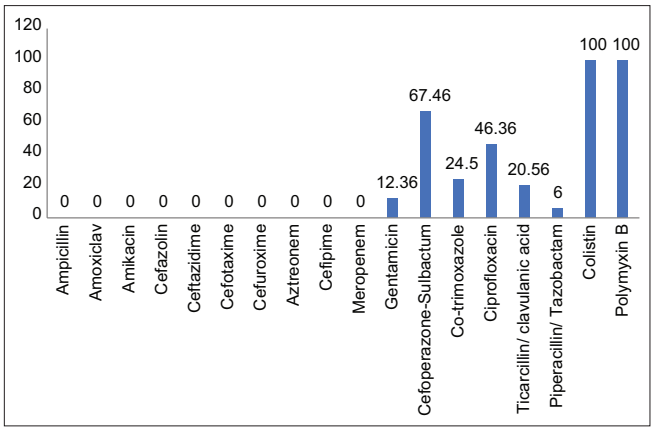

Figure 8: Distribution antibiotic susceptibility of Carbapenem resistant isolates (%)

pneumonia (with COPD), 1 was immunocompromised with respiratory failure, and COPD died in the study.

Discussion

Ps. aeruginosa is a prevalent pathogen in nosocomial infections, especially among critically ill patients. Isolates have appeared as an issue of great concern to the identification of MBL-producing strains in hospital.[13] As per the availability of phenotypic tests, the bacterial isolates escape from routine antibiotic susceptibility testing in the laboratory.[14] In the study, the surgical ward found a maximum number of bacterial isolates than the medical and other wards. Fifty six pus specimens were found maximum carbapenem resistant (32.14%) than other specimens [Figure 4]. Collected specimens from patients can be easily correlated with the site of infection. For examination of respiratory infection in patients, collected the sample from the medicine department by aspiration and endotracheal tube. From surgery, patient cases collected the specimen was found with ulcerative and nonulcerative lesions, diabetic ulcers, and traumatic ulcers. The study found a similar finding as other studies found frequently *Ps. aeruginosa* in suppurative skin infections and respiratory infections.[15] Bacterial isolates were found predominantly among males (61.5%) and the 0-65 years age group with the 41-50 years age group of patients with *Ps. aeruginosa* infection was prominent, whereas patients with MBL-*Ps. aeruginosa* infection had 41-50 years age group of patients much higher than the reported as another age group commonly.[16,17]

In this study, all 39 isolates were found resistant to meropenem, 32 (82.05%) bacterial isolates were resistant to both meropenem and imipenem, and 7 (17.95%) isolates were found resistant to meropenem but sensitive to imipenem.

All meropenem and imipenem-resistant 32 bacterial strains were uniformly sensitive to polymyxin B and colistin. As these are peptides in nature[18] and are useful as the last resort for the therapeutic drug of choice, MBL-producing *Ps. aeruginosa* were additionally found resistant to aztreonam.[15] Due to high incidence of nephrotoxicity and its association with this drug limits the use of this drug in the treatment of patients.[19]

The study randomly selected 20 bacterial strains which were resistant to both (meropenem and imipenem) also tested for the minimum inhibitory concentration (MIC) antibiotic susceptibility testing by E-test for imipenem and meropenem and found 15 isolates resistant with >16 ug/mL concentration for both but of 20 bacterial isolates, 5 bacterial isolates showed resistant with >16 ug/mL concentration for meropenem; only these 5 bacterial strain were found sensitive with <4 ug/mL concentration for imipenem.[21]

In our study, MHT showed higher positivity for carbapenem-resistant strains. Carbapenem-resistant strain screened were for MBL production and found maximum by MHT test showed positive with meropenem (76.92%) and imipenem (71.79%). Another study defined that the MHT test is used to detect carbapenems activity but it does not give the confirmation of the carbapenems as a phenotypic test due to lower sensitivity or specificity. As per Behera *et al*.[20] recorded equity of test performance for the CDST and E-test. As per some investigators, CDST[10,22] is satisfactory but our study recorded higher specificity for DDST for the detection of MBL production with carbapenem drugs. Other investigators' study recorded the MHT test with higher specificity for carbapenemase production, so the study was recorded as less reliable than DDST. DDST has less channel of subjective variation, which measures the enhancement of the zone of inhibition at the standard distance of both discs as per standard guidelines of testing but CDST interprets more. Subjective but both tests are limited in terms of temperature, aeration,

pH and thickness of media, and disc potency which may vary with the individual discs. As per our study, test procedures showed that the procedures used for the detection of carbapenem resistance are not statistically significant [Table 1] [calculated *P* value is. 12 (*P* value >.05)]. So the study requires to do with more numbers of carbapenem-resistant strains specifically to be statistically significant.

However, the test between meropenem and meropenem with EDTA disc enhanced by diffusion method demonstrates the better synergy test; effective chelation EDTA concentration must show the diffusion at a standard distance of disc meropenem (also for imipenem) which may differentiate the results of CDST and DDST found in our study.

In our study, 39 carbapenem-resistant strains in male patients (61.54%) were found a number of cases more than in female patients (38.46%), which explained the cases with a higher prevalence in the surgical department (46.15%) followed by ICU (28.21%), orthopedic (15.38%), TB and Chest (7.69%), and ENT (2.56%) and in other studies found in the surgical wards (6.06%) and in ICU (22.4%).[23,24] In another study, surgical department found the highest prevalence may be due to the patient attended by another hospital and was on third-line medication of cephalosporins. ICU patients reported mortality but other clinical department patients reported only morbidity. In another study, mortality was found higher than in our study.[23] This may be due to higher comorbidity, invasive procedures, nonhealing ulcers after surgery, longer stay in the hospital, and excessive use of broad-spectrum antibiotics.

As multidrug-resistant *Ps. aeruginosa* now-a-days[25] and reported with mortality and morbidity. As per the reference micro-organism resistant to three or more antibiotic classes as beta-lactamase, aminoglycosides, and fluoroquinolones is considered MDR in *Pseudomonas*.[26] Our study identified MDR *Ps. aeruginosa* and of which 20 were MBL-producing and it also showed a significant association; it may be inclusive due to the small sample size. Several factors showed the association with MBL-PA infections.[13] But in our study, association of risk factors statistically were not significant for carbapenem-resistant strains. Other risks as prior antibiotic use, hospitalization >8 days, and endotracheal intubation in ICU patients similar to the prior studies recorded that the prior exposure of beta-lactam or fluoroquinolone, urinary tract infection, renal failure, and ICU stay significantly considered as risk factors for MBL-PA.[21] As per previous studies found patients which were exposed to antibiotics, especially ciprofloxacin, metronidazole, and cefotaxime.[24]

Table 1: Carbapenem drug resistance with two test procedures

Antibiotics	By Kirby Baur Disc Diffusion Method (Positive)	By Modified Hodge test (Positive)
Meropenem	39	30
Imipenem	32	28

Statistically procedure for detection of carbapenem resistant is not significant. [calculated *P* value is 0.12 (*P*>0.05)]

The limitation of the study was the short study duration and small sample size so the observation of the study might be affected by economically weaker patients, seasonal trends of patient admission, and rural area tertiary healthcare centers awaiting improvement of facility which may associate admission avoidance by the patient. With so, evaluation still needs to conclude a positive association as per our study. E-test–based testing was not done in all isolates.

Conclusion

MBL production identified the resistant mechanism of resistance to the carbapenem group of drugs including another group of antibiotics. Prevalence was found to be similar to other studies but the resistance to another group of antibiotics was found lower. Also, disc diffusion method was reliable for screening with a correlation of DDST or MHT. Our study was focused to improve hospital infection control by the prevalence of organisms and their related risk factors. Risk factors correlation is important for the standard treatment procedure, which may help in the care and treatment of patients after admission.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Poirel L, Naas T, Nicolas D, Collet L, Bellais S, Cavallo JD, *et al*. Characterization of VIM-2 a carbapenem – hydrolyzing, metallo- Beta lactamases and integron- borne gene from a Pseudomonas aeruginosa clinical isolate in France. Antimicrob Agents Chemother 2000;44:891-7.
2. Ramakrishnan K, Rajagopalan S, Nair S, Kenchappa P, Chandrakesan SD. Molecular Characterization of MBLs producing MDR Pseudomonas aeruginosa from various clinical samples. Indian J Pathol Microbiol 2014;57:579-82.
3. Rossolini GM, Mantengoli E. Treatment and control of severe infections caused by multiresistant Pseudomonas aeruginosa. Clin Microbio Infect 2005;11:17-32.
4. Landman D, Bratu S, Kochar S, Panwar M, Trehan M, Doymaz M, *et al*. Evaluation of microbial resistance among Pseudomonas aeruginosa, Acinetobacter baumannii and Kleb.

Pneumoniae in Brooklyn, NY. J Antimicrob Chemother 2007;60:78-82.

5. Noyal MJC, Menezes GA, Harsih BN, Sujotha S, Parija SC. Simple screening tests for detection of carbapenemases in clinical isolates of non-fermentative Gram–Negative bacteria. Indian J Med Res 2009;129:707-12.
6. Yan JJ, Hsueh PR, Chien Ko W, Tay Luh K, Tsai SH, Mei Wu H, *et al*. Metallo β lactamase in clinical Pseudomonas aeruginosa Isolates in Taiwan and identification of VIM-3, a novel variant of the VIM-2 enzyme. Antimicrob Agents Chemother 2001;45:2224-8.
7. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI Supplement M100. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2020. p. 40.
8. Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of Hodge test and the imipenem-EDTA double-Disc synergy test for differentiating metallo-ß-lactamase producing isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Microbiol 2003;41:4623-9.
9. Agrawal G, Lodhi RB, Kandakar UP, Khadese RK, Jalgaonkar SV. Study of Metallo beta Lactamase production in clinical isolates of Ps. aeruginosa. Indian J Med Microbiol 2008;26:349-51.
10. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem EDTA Disc method for differentiation of metallo-β-lactamases producing clinical isolates of Ps. spp and Acinetobacter spp. J Clin Microbiol 2002;40:3798-801.
11. Lee K, Chong Y, Shin HB, Kim YA, Yong D, Yum JH. Modified hodge and EDTA-disc synergy tests to screen metallo-lactamase-producing strains of Ps. and Acinetobacter species. Clin Microbiol Infect 2001;7:88–91.
12. Ramana KV, Rao R, Sharada CV, Kareem MA, Reddy LR, Mani R. Modified Hodge test: A useful and the low-cost phenotypic method for detection of carbapenemase producers in Enterobacteriaceae members. J Natural Sci Biol Med 2013;4:346-8.
13. De AS, Kumar SH, Saveja SM. Prevalence of metallo-beta Lactamase producing Ps. aeruginosa and Acinetobacter spp. in Intensive care areas in a tertiary care hospital. Indian J Crit Care Med 2010;14:217-9.
14. Franklin C, Liotios L, Peleg AY. Phenotypic calefaction of carbapenems-susceptible metallo-Beta Lactamases-producing gram-negative bacilli in clinical laboratory. J Clinical microbial 2006;44:3139-44.
15. Zavascki AP, Barth AL, Gaspareto PB, Saraiva Goncalves AL, Didonet Moro AL, Fernandes JF, *et al*. Risk factors for nosocomial infection due to Ps. aeruginosa producing metallo-beta lactamase in two tertiary-care teaching hospitals. J Antimicrob Chemother 2006;58:882-5.
16. Marra AR, Pereira CAP, Gales AC, Menezes LC, Cal RGR, De Souza JMA, *et al*. Blood stream infections with metallo-beta lactamase producing Ps. aeruginosa epidemiology, microbiology and clinical outcomes. Antimicrob Agents Chemother 2006;50:388-90.
17. Tsakris A, Poulou A, Kristo I, Pittaras T, Spanakis N, Pournaras S, *et al*. Large dissemination of VIM-2-Metolla-beta-Lactamase producing Ps. aeruginosa strains causing health care-associated community-onset infections. J Clin Microbial 2009;47:3524-9.
18. Storm DR, Rosenthal KS, Swanson PE. Polymyxin and related peptide antibiotics. Annu Rev Biochem 1977;46:723-63.
19. Falagas M, Kasiakou S. Toxicity of polymyxins: A systematic review of the evidence from old and recent studies. Crit Care 2006;10:R27.
20. Behera B, Mathur P. High levels of antimicrobial resistance at a tertiary trauma care centre of India. Indian J Med Res 2011;133:343-5.
21. Shanthi M, Sekar U. Multi-drug Resistant Pseudomonas aeruginosa and Acinetobacter baumannii infections among hospitalized patients: Risk factors and outcomes. J Assoc Physicians India 2009;57:636-40.
22. Berges L, Rodriguez VH, Deplano A, Struelens MJ. Prospective evaluation of imipenem/EDTA combined disc and E-test for detection of metallo-beta-lactamase producing Pseudomonas aeruginosa. J Antimicrob Chemother 2007;59:812-3.
23. Kumar SH, De AS, Baveja SM, Gore MA. Prevalence and risk factors of Metallo beta lactamage producing Pseudomonas aeruginosa and Acinetobacter species in burns and surgical wards in a tertiary care hospital. J Lab Physicians 2012;4:39-42.
24. Kali A, Srirangaraj S, Kumar S, Divya HA, Kalyani A, Umadevi S. Detection of Metallo Beta lactamase producing Pseudomonas aeruginosa in intensive care units. Australas Med J 2013;6:686-93.
25. Jahn S, Balagurunathan R. Metallo Beta Lactamases producing Ps. aeruginosa and Acinetobacter baumannii. Indian J Med Microbiol 2011;29:302-4.
26. Hammani S, Bautiba-ben Boubaker I, Ghozzi R, Saidani M, Amine S, Redjeb SB. Nosocomial outbreak of imipenem-resistant Ps. aeruginosa producing VIM-2 metallo-beta lactamase in a kidney transplantation unit. Diagn Pathol 2011;6:106.