Original Research Article

Detection of Metallo-β-lactamase producing Gram Negative Bacteria in clinical isolates in Tertiary care Hospital - A prospective study

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Abstract

Background: Carbapenem resistance in Gram Negative Bacilli is an emerging threat in tertiary care centers which is mediated by Metallo-β-lactamase (MBL) enzyme. As per the National committee for Clinical Laboratory Standards (NCCLS), still does not have documented standard procedure from there several screening methods to detect their enzyme. Some subcontinents of India still awaiting to see prevalence and screening methods to detect enzyme which is responsible for Carbapenem Resistance.

Aim: The present study was undertaken to early detection of MBL by screening methods in Gram Negative Bacilli isolated from hospital and the prevalence MBL production in carbapenem resistant bacterial isolates.

Materials and methods: 176 consecutive different Gram Negative Bacilli (GNB) isolated from hospitalized patients which were tested antimicrobial susceptibility for different antibiotics including Carbapenem drugs as Imipenem by Kirby Bauer Disc Diffusion (CLSI 2010) and screening of Metallo-β-lactamase production by method as Imipenem- EDTA combined disc synergy test (I-CDST) and Imipenem-Double Disc Synergy Test (I-DDST) which determine the MBL by zone size enhancement with EDTA Impregnated Imipenem.

Results: Out of 176 Gram Negative Bacilli, 20.45% (n=36) of isolates were resistance to Imipenem by disc diffusion method and 94.44% (n=34) by DDST EDTA impregnated Imipenem and 88.89% (n=32) showed enhancement of zone size ≥7 mm with EDTA impregnated Imipenem CDST. Imipenem susceptible bacteria strains did not show any enhancement with EDTA impregnated antibiotic disc.

Conclusion: Critically ill patient's therapy is cause of concern for MBL mediated imipenem resistance gram Negative Bacilli. Two methods used for supplementary support in treatment of patients. In both methods of detection DDST is more effective.

Key words

Carbapenems- Imipenem, Metallo-β-lactamase, Gram Negative Bacilli, EDTA-DDST.

Introduction

Carbapenem, β-lactam drug were great advancement for treatment in clinics [1]. In 1960, *Bacillus cereus* first identified MBL and in *Pseudomonas aeruginosa* in 1991. Since 1990, genes encoding MBL have been reported worldwide as members of Enterobacteriaceae, *Pseudomonas spp* and *Acinetobacter spp* [2]. Acquired MBL isolates have emerged due to resistance mechanisms with hydrolysis of all β-lactam drugs including carbapenems [3]. Plasmids encoded spread of MBLs isolates developed infection as nosocomial including outbreaks in admitted to ICUs with different morbidities and antibiotics use [4]. The isolates were related with morbidity and mortality of patients [5]. Early detection of MBL-producing is crucial to establish infection control measures and appropriate therapy to prevent dissemination from MBLs producers to other GNB [6] and their spread in interhospital and intrahospital [7]. MBL Detection by CDST and DDST method developed for routine laboratories [8, 9]. So, study focused on to effective screening test for detection and to know the prevalence of the MBL in various GNB isolates.

Materials and methods

A prospective study was conducted over a period of May 2015 to June 2016 at Department of Microbiology and received 176 Gram Negative Bacilli isolates from different clinical specimens in a tertiary care hospital and admitted patients included for study after exclusion of out patients. The study was approved by institutional ethics committee. Out of 176 isolates were 36 isolates Carbapenem resistant. These organisms were isolated from specimens as urine, pus, sputum, blood, otitis media, pleural fluid, tracheal aspirate and ascitic fluid of admitted patients. Specimens were processed in department of microbiology for routine identification and antibiotic susceptibility testing.

Different Gram Negative Bacterial species identified as per standard procedures [10]. MBL screening methods: Antimicrobial sensitivity was performed on Mueller Hinton Agar (Himedia) by Kirby Bauer Disc Diffusion Method according to the clinical and laboratory Standard Institute Guidelines (CLSI, 2010) [11].

MBL confirmatory Test: For MBL detection confirmatory test done by using Imipenem-EDTA double disc synergy test (IPM-DDST) and CDST (Imipenem- EDTA combined disc synergy Test) methods [2].

Imipenem -EDTA Combined disc synergy test (CDST- Imipenem): Disks of Imipenem (10µg, Himedia) and Imipenem with ethylene diamine tetraacetic acid, (EDTA) (10µg + 750 mg, prepared in house) for MBL detection were used. Inoculated plates were incubated for 16-18 hours at 37 ºC. If the increase in inhibition zone with Imipenem - EDTA disc was ≥7 mm than the Imipenem disc alone then it was considered as MBL positive.

Imipenem -EDTA Double disc synergy test (DDST- Imipenem): A Imipenem (10ug) disc

was placed 20 mm center to center from a blank disc containing 10ul of 0.5M EDTA (750ug). Inoculated plates were incubated for 16-18 hours at 37ºC. If enhancement in zone of inhibition between Imipenem and EDTA disc which was considered as positive for MBL production.

Results

In the study, out of 176 Gram Negative Bacilli isolates were highest in urine 68 (38.64%). Out of 36 (20.45%) carbapenem resistant isolates in urine were identified highest 12 (33.33%), shown in **Table - 1**. *Escherichia coli* showed highest prevalence 12(33.33%) follow *Klebsiella pneumoniae* 7(19.44%), *Acinetobacter spp.* 6 (16.67%), *Pseudomonas aeruginosa* 4 (11.11%), *Klebsiella oxytoca*, *Proteus mirabilis* and other Gram Negative Bacilli 2 (5.56%), *Citrobacter freundii* 1(2.78%) as shown in **Table – 2**. Imipenem antimicrobial agent used in which 32 (88.89%) isolated were shown MBL positive by using CDST method and 34 (94.44%) isolates were identified positive by DDST-IPM with which was negative by CDST-IPM as shown in **Table - 2**. After the repetition of procedure, it produced similar and reproducible zone diameters. Comparison of our study was as per **Table – 3**.

Table - 1: Sample wise distribution of clinical isolates with Carbapenem resistance.

Specimens	**Clinical Isolates no. (%)**	**Carbapenem Resistant isolates no. (%)**
Urine	68 (38.64)	12 (33.33)
Pus	52 (29.55)	9 (25)
Sputum	29(16.48)	7 (19.44)
Blood	18(10.23)	4 (11.11)
Foleys Cathe. Tip	4(2.27)	2 (5.56)
ET tip	2(1.14)	1 (2.78)
Fluid	2(1.14)	1 (2.78)
Otitis media	1 (0.57)	0
Total	**176**	**36**

Table - 2: Carbapenem Resistant isolates with difference between MBL detection tests.

Microorganism	**Carbapenem Resistant Isolates n(%)**	**MBL detection test**	
		By DDST n=34 (%)	**By CDST n=32 (%)**
Escherichia coli	12 (33.33)	11(32.35)	10 (31.25)
Klebsiella pneumoniae	7 (19.44)	7 (20.59)	6 (18.75)
Acinetobacter baumannii	6 (16.67)	5 (14.71)	5 (15.63)
Pseudomonas aeruginosa	4 (11.11)	4 (11.76)	4 (12.5)
Klebsiella oxytoca	2 (5.56)	2 (5.88)	2 (6.25)
Proteus mirabilis	2 (5.56)	2 (5.88)	2 (6.25)
Other GNB	2 (5.56)	2 (5.88)	1 (3.13)
Citrobacter freundii	1 (2.78)	1(2.94)	1 (3.13)

Table - 3: Comparison in Percent of present study with other published studies.

Study	**CDST-IPM (%)**	**DDST-IPM (%)**
Galani, et al. [12]	94.7	100
Picao, et al. [7]	80	82.6
Franklin, et al. [13]	100	79
Nirav P Pandya [2]	96.30	81.48
Present study	88.89	94.44

Discussion

In our study, antimicrobial drugs resistance also found for other antibiotics. As per the reported studies there are no standard guidelines for detection of MBLs but the molecular method is sensitive and specific for detection of MBL but it is not feasible in routine microbiology laboratory. For MBL detection in 36 isolates out of 176 Gram Negative Bacilli isolates where DDST-IPM was most sensitive (94.44%) followed to CDST-IPM (88.89%). Interpretation of CDST-IPM results may be depends upon the technical experts to see true synergism. Carbapenem resistant Gram negative Bacilli with prevalence of MBL production DDST-IPM method was found *Escherichia coli* showed highest prevalence 33.33%. In the other studies, DDST-IPM found most sensitive for detection of MBL production GNB, contrasting to present study and the other studies found CDST-IPM as the least sensitive method similar to present study, which shown in table no.3. Interpretation of CDST method is more subjective as it may depends upon the good technical practice for the procedure of determine the true synergism with antibiotic disc composition.

Prevalence of MBL production in DDST-IPM method found highest in *Escherichia coli* 11(32.35%) and MBL production was also detected decreasingly by percent in *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Proteus mirabilis* and other Gram Negative Bacilli, *Citrobacter freundii*. MBL positive strains show resistance to all β-lactam antimicrobial agents including aminoglycosides, fluoroquinolones. However they remain sensitive to Colistin and Polymyxin B.

Conclusion

In the study finding show that there are significant numbers of Gram Negative isolates MBL producing. There is a need to detect MBL producers with active surveillance and by use of early detection method. Early detection of isolates is helpful to reduce the mortality and morbidity for the patients in hospital. Environment where the MBL strain dissemination chance is more. And the DDST-IPM is more convenient screening method for detection of MBL producing Gram Negative Bacilli in routine microbiology laboratory. This may help to clinician to follow antibiotic to follow antibiotic restriction policies with excessive use of carbapenems and other brand spectrum antibiotics.

References

1. Jesudason MV, Kandathil AJ, Balaji V. Comparison of two methods to detect carbapenemase and metallo-β-lactamase production in clinical isolates. Indian J Med Res., 2005; 121: 780-3.
2. Pandya NP, Prajapati SB, Mehta SJ, Kikani KM, Joshi PJ. Evaluation of Various methods for detection of metallo-β-lactamase (MBL) production in Gram Negative Bacilli. Int J Biol Med Res., 2011; 2(3): 775-777.
3. Behera B, Mathur P, Das A, Kapil A, Sharma V. An evaluation of four different phenotypic techniques for Detection of metallo-β-lactamase producing *Pseudomonas aeruginosa*. Ind J Med Microbiol., 2008; 26(3): 233-7.
4. Maltezou HC. Metallo-β-lactamases in Gram Negative Bacteria: introducing the era of pan-resistance? Int J Antimicrob Agents, 2009; 33(5): 405.
5. Dwivedi M, Mishra A, Azim A, Singh RK, Baronia AK, Prasad KN, Dhole TN, Dwivedi UN. Ventilator-associated pneumonia caused by carbapenem-resistant Enterobacteriaceae carring multiple metallo-β-lactamase genes. Ind J Path Microbiol., 2009; 52(3): 339-342.
6. Deshmukh DG, Damle AS, Bajaj JK, Bhakre JB, Patwardhan NS. Metallo-β-lactamase producing clinical isolates from patients of a tertiary care hospital. J Laboratory Physicians, 2011; 3(2): 93-97.

7. Picao RC, Andrade SS, Nicoletti AG, Campana EH, Moraes GC, Mendes RE, et al. Metallo-β-lactamase detection comparative evaluation of double disk synergy versus combined disk tests for IMP, GIM, SIM, SPM or VIM-producing isolates. J Clin Microbiol., 2008; 46(6): 2028-37.
8. Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge test and the imepenem-EDTA double-disk synergy test for differentiating metallo-β-lactamase producing isolates of *Pseudomonas spp.* and *Acinetobacter spp*. J clin Microbial., 2003; 41: 4623-9.
9. 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.
10. Konman EW, Allen AD, Janda WM, Schreckenbenger PC, Winn WCJR. Collection, transport, processing, analysis and reporting of cultures from specific specimen sources. In Color atlas and textbook of diagnostic microbiology, 6th edition. Lipincott Williams and Wilkins, p. 68-105.
11. Clinical and Laboratory Standard Institute. Performance standards for antimicrobial susceptibility testing; Twentienth information supplement, document M100-S20. Warne, PA: Clinical Laboratory Standard Institute; 2010, 45-110.
12. Galani I, Rekatsina PD, Hatzaki D, Plachouras D, Souli M, Giamarellou H. Evaluation of different laboratory tests for the detection of metallo-β-lactamase production in Enterobacteriaceae. J Antimicrob Chemother., 2008; 61: 548-53.
13. Franklin C, Liolios L, Peleg AY. Phenotypic Detection of Carbapenem-Susceptible Metallo-β-lactamase-Producing Gram-Negative Bacilli in the Clinical Laboratory. J Clin Microbiol., 2006; 44(9): 3139-44.