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Evaluation of Diagnostic Test in Emerging Carbapenem Resistant Gram Negative Bacilli in Patients admitted to Tertiary Care Centre in North India

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A B S T R A C T

Carbapenem antibiotics are very often used against multidrug resistant strains clinically troublesome pathogens which developed and proved that the resistance and metallo-β-lactamases (MBL) production were a disaster in treating infections. The identification and detection of MBL-producing bacterial strains were having crucial importance for the prevention of nosocomial infections. Therefore the present study was undertaken for screening MBL production Gram Negative bacteria. One hundred twenty two 122 consecutive Non-repetitive isolates of gram negative bacilli clinical isolates were subjected to susceptibility testing by disc-diffusion test on Mueller Hinton Agar. Meropenem resistant (MR) strains MBL production among MR stains were further screened by Meropenem- EDTA combined disc synergy test (M-CDST) and Meropenem-EDTA double-disc synergy test (M-DDST). A total of 31 isolates showed resistance to Meropenem which were screened and 29 (93.55%) isolates gave positive result by M-DDST whereas 27 (87%) were MBL producers by M-CDST. Escherichia coli isolates recorded highest as MR strains were identified. For the treatment, implementation of effective infection control and prevention of nosocomial dissemination used the procedure for detection and identification of carbapenem resistant by most reliable method for study of MBLs produced isolates. The more effective method was M-DDST in comparison of other method as M-CDST.

Introduction

The emergence of carbapenem resistant strains among gram negative bacteria is a notable threat. Clinically relevant bacterial species detected often resistant to different β-lactam antibiotics, including the antibiotics which cover extended spectrum cephalosporins, but rarely to carbapenems (Chu *et al.*, 2001). Among the B-lactams drugs, carbapenems were potent agents for treatment of serious infections by gram-negative bacteria. Their broad spectrum activity and resistance to hydrolysis by most B-lactamases, including the extended-spectrum B-lactamases (ESBL) (Bush *et al.*, 1995). Carbapenems antibiotics are the drug of choice for treatment of extended spectrum

beta lactamase (ESBL) producing gram negative bacterial infections where the penicillin and other group cephalosporin antibiotics were resistant. Resistant Gram negative bacterial isolates are the important causative agents for urinary tract infections, bloodstream infections, healthcare-associated pneumonia, intra-abdominal infections and ventilator associated pneumonia. The increasing resistance of Carbapenem antibiotics in family Enterobacteriaceae and other group of Gram negative bacilli were a significant challenge with increasing prevalence. So Gram negative bacteria recognized resistance against different group of antibacterial drugs Worldwide. (NNIS, 2004)

However, in past few years resistance to carbapenems due to production of carbapenemases have been reported. Carbapenemases may be defined as beta-lactamases that significantly hydrolyze at least imipenem or meropenem. Most significant involved in acquired resistance are of Ambler molecular classes A, B and D. Class B or the Metallo–beta-lactamases (MBLs) enzymes are the most significant carbapenemases (Nordmann and Poirel, 2002; Deeba Bashir *et al.,* 2011; Walsh *et al.,* 2005). Carbapenemases especially MBLs due to transferrable in character are the most feared because it can hydrolyse almost all antibiotics including carbapenem antibiotics. So the study was conducted the detection of the carbapenem antibiotics resistance in gram negative bacilli isolates from different clinical specimens by phenotypic methods which may help to screen the population in hospital environment to guide effective empirical therapy.

Materials and Methods

A prospective study was carried out on 122 non repetitive gram negative bacilli isolates which has been isolated various clinical specimens from hospitalized patients. The study was approved by institutional ethics committee. The isolates were identified as per standard conventional methods as per CLSI guidelines 2010 (CLSI, 2010) in which incorporated the antibiotics were ampicillin (10 mcg), amocicillin/clavulenic acid (30 mcg) gentamicin (10 mcg), amikacin (30 mcg), netilmicin (10 mcg), cefotaxime (30 mcg), ceftriaxone (30 mcg), ceftazidime (30 mcg), cefepime (30 mcg), CIprofloxacin (5 mcg), meropenem (10 mcg), cefoperazone/ sulbactam (75/10 mcg), piperacillin/ tazobactum (100/10 mcg) with polymyxin B (300 Units), tigecycline (15 mcg) and these were tested for *in-vitro* Carbapenem resistance and then tested to see MBL production in the bacterial isolates.

Isolated Gram negative bacilli identified from different clinical specimens which were resistant to carbapenem group of antibiotic as Meropenem. The gram-negative bacilli were showing the resistance for carbapenem antibiotic on routine screening was confirmed for presence of MBL production. Briefly, Muller-Hinton agar used for antibiotic susceptibility testing.

The combined disc and double disc synergy test methods were used to confirm above resistance mechanisms for MBL production (Lee *et al.,* 2003).

Meropenem-EDTA combined disc synergy test (CDST-Meropenem)

Disks of Meropenem (10mcg, Himedia) and Meropenem with ethylene diamine tetraacetic acid, (EDTA) (10mcg + 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 Meropenem- EDTA disc was ≥7 mm than the Meropenem disc alone then it was considered as MBL positive.

Meropenem-EDTA Double Disc Synergy Test (DDST-Meropenem)

A Meropenem (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 Meropenem and EDTA disc which was considered as positive for MBL production.

Results and Discussion

A total of 122 consecutive Non-repetitive isolates of gram negative bacilli obtained from various clinical samples were included in the study out of which 46 were isolated highest from pus, 32 follow urine, 21 sputum, 15 blood, 3 Urine Catheter tip, 2 Endotracheal Tube, 2 fluids and 1 from otitis media as depicted in Table 1.

Out of 122 Gram Negative Bacilli isolates were highest in pus 46 (37.7 %). Out of 31 carbapenem resistant isolates in pus identified highest isolates 11 (35.48%), shown in Table 1. In 31 carbapenem resistant isolates, highest isolates were recorded in surgical ward 12(39%), followed medicine 7(23%), ICU 5(16%), OBS/Gynae 4(13%), Orthopedic 2(6%) and Paediatric 1(3%) shown in Chart no 1. Antimicrobial susceptibility in MBL producing bacterial strains showed resistant to different antibiotics as group of cephalosporins, aminoglycosides, fluoroquinolones, carbapenem drug as meropenem, and seen 100% sensitive for Polymyxin B, colistin followed 46% Tigecycline which were showed in Table 2. In Gram Negative Bacilli out of 31 carbapenem resistant highest isolate was *Escherichia coli* 8(25.81%) followed *Pseudomonas aeruginosa* and *Acinetobacter baumannii* 6(19.35%), *Klebsiella pneumoniae 4(12.9%)*, *Klebsiella oxytoca* 2(6.45%), *Proteus mirabilis* 2(6.45%), Other GNB 2(6.45%) and *Citrobacter freundii* 1(3.23%). MBL detection test showed positive, for MBL production higher by DDST (29 isolates) and by CDST (27 isolates), shown in Table 3.

Infections caused by multidrug resistant gram negative bacterial where Carbapenem antibiotic proved most potent agents for treatment. MBL production is a most important mechanism to hydrolyse the Carbapenem antibiotics which emerged as the Carbapenem resistance. As per the therapeutic significance these bacterial isolates in study were also showing resistance for many other antibiotic groups like beta-lactams, aminoglycosides, fluoroquinolones and out of these, options left for therapy are use of Polymixin B and Colistin antimicrobial agent which carry potential toxicity (Gupta *et al.,* 2012; Jesudason *et al.,* 2005; Gupta *et al.,* 2006). In the study highest number of resistance strains found from surgical department as similar (Nagaraj *et al.,* 2012) except the other found in intensive care unit (Gupta *et al.,* 2006; Mahajan *et al.,* 2011; Sinha *et al.,* 2007). The continuation in increasing prevalence of MBL producing strains has proved to be a clinical disaster and due to unnoticed spread within hospital or institution may turn to serious challenge for infection control management. And MBL producing strains may participate in horizontal MBL gene transfer to other pathogens in the hospital settings due to intrinsic capability of MBL producing strains. As Early detection of MBL producing bacteria in infections is need to treat appropriate with in time limit which might reduce the mortality when patient stay in hospital (Arakawa *et al.,* 2000). MBL producing strain screening methods had been employed in different studies but due to no standard guidelines CLSI for detection of MBL which not laid Performance standards (Behera *et al.,* 2008). In the present study we had used two conventional phenotypic tests

for detection of MBL production as Meropenem-EDTA Combined Disc Test (CDST) and Meropenem-EDTA Double Disc Synergy Test (Meropenem-DDST). Although to see the Meropenem resistance by E test is also used for MBL detection but CDST and DDST are comparable to it and are also simple, reliable, inexpensive and reproducible (Yan *et al.*, 2004). We had found that with Meropenem-EDTA DDST, the positives and negatives properly but with CDST it may be due to subjective variations with calculation for preparation of standard reagents. DDST identification was done with discriminating the true synergism. So, the DDST method using Meropenem-EDTA had good impact over CDST. As per the finding is in accordance with other studies which had found DDST to be one of the most sensitive technique for detecting MBL in comparison of CDST.

Table.1 Sample wise distribution of clinical isolates with carbapenem resistance

Specimens	Clinical Isolates [no. (%)]	Carbapenem Resistant isolates [no. (%)]
Pus	46 (37.7)	11(35.48)
Urine	32 (26.23)	8 (25.81)
Sputum	21(17.21)	5(16.13)
Blood	15(12.3)	3(9.68)
Urine Catheter Tip	3(2.46)	2(6.45)
ET tube	2(1.64)	1(3.23)
Fluid	2(1.64)	1(3.23)
Otitis media	1 (0.82)	0
Total	**122**	**31**

Table.2 *In vitro* available susceptibility of MBL and Non MBL producing GNB isolates

Antibiotics	Number of Non MBL Strain (n=122)	Percentage of MBL Strains (n=36)
Ampicillin	30	0
Amoxicillin/Clavulanic Acid	78	0
Cefotaxime	112	0
Ceftriaxone	114	0
Ceftazidime	108	0
Cefepime	116	0
Piperacillin/Tazobactam	120	0
Gentamicin	94	0
Amikacin	114	0
Neticillin	94	0
Ciprofloxacin	114	0
Cefoperazone/Sulbactam	92	0
Meropenem	122	0
Tigecycline	122	46
Colistin	122	100
Polymyxin B	122	100

Table.3 Carbapenem resistant isolates with difference between MBL detection tests

Microorganism	Carbapenem Resistant Isolates	MBL detection test	
		By DDST [n=29 (%)]	By CDST [n=27 (%)]
Escherichia coli	8 (25.81)	8(27.59)	7 (23.93)
Pseudomonas aeruginosa	6 (19.35)	6(20.69)	6 (22.22)
Acinetobacter baumannii	6 (19.35)	5(17.24)	5(18.52)
Klebsiella pneumoniae	4 (12.9)	4(13.79)	3 (11.11)
Klebsiella oxytoca	2 (6.45)	2(6.9)	2(7.41)
Proteus mirabilis	2 (6.45)	2(6.9)	2(7.41)
Other GNB	2 (6.45)	1(3.45)	1(3.70)
Citrobacter freundii	1 (3.23)	1(3.45)	1(3.70)
	31		

Chart.1 Ward-wise distribution of Meropenem resistant 31 Gram Negative bacterial isolates

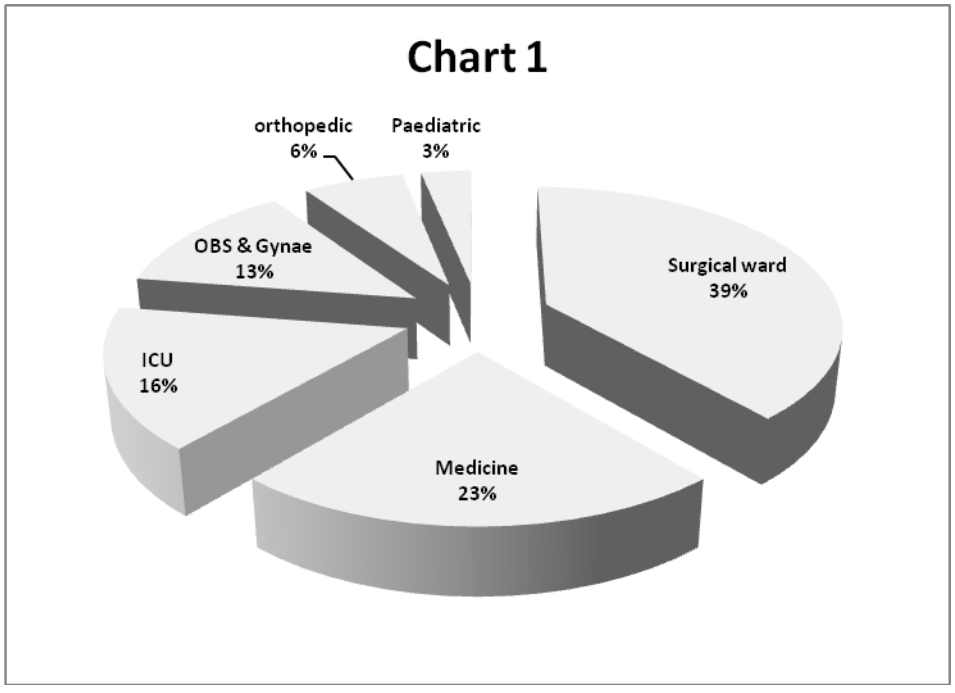
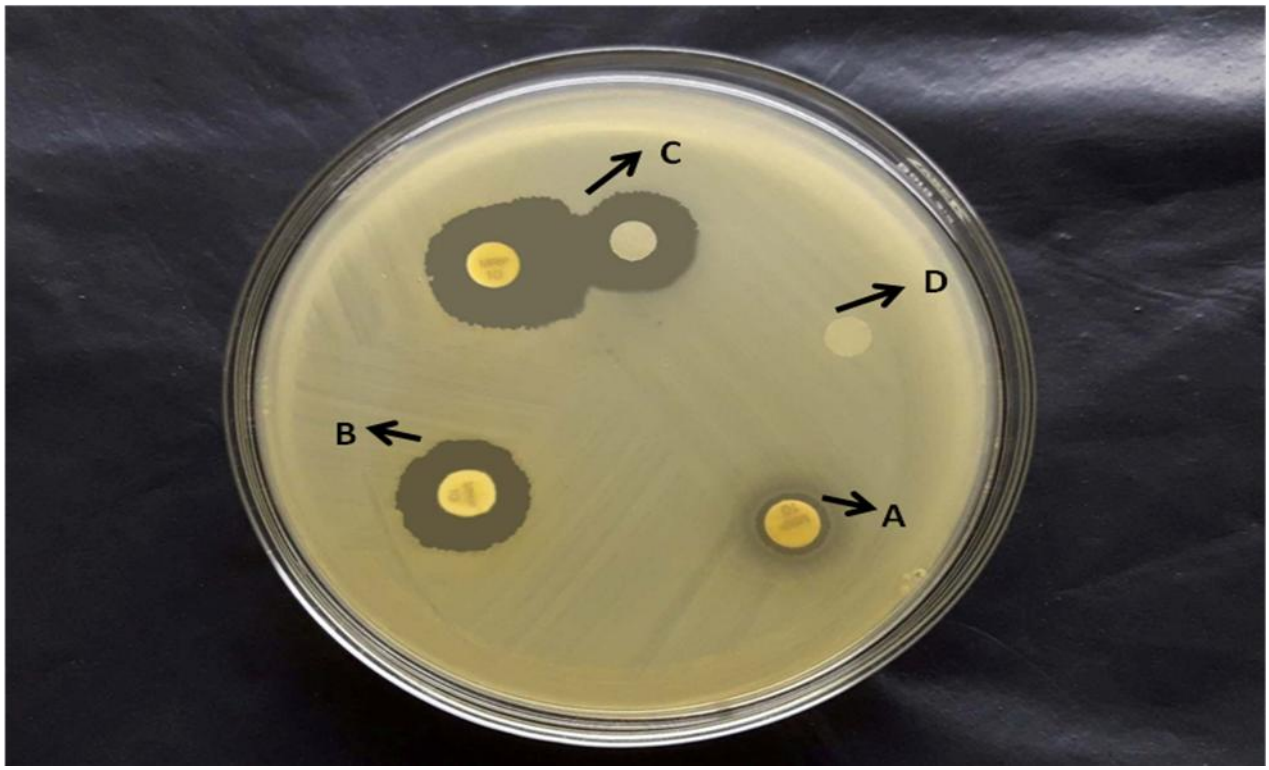


Fig.1 Showing antibiotic susceptibility testing in phenotypic method of Meropenem resistant strains for detection of metallo-β-lactamase producers. (A) Meropenem antibiotic resistant (B) Showing Combined Disc Synergy Test (Meropenem antibiotic incorporated EDTA disc) (C) Showing Double Disc Synergy Test (one disc Meropenem and other disc with EDTA) (D) Showing Blank disc



In India prevalence ranging from 14 to 20% has been reported in studies Meropenem-DDST identified most sensitive test for detection of MBL production and hence Meropenem disc is a better option for screening MBL (Sinha *et al.,* 2007; Sinha *et al.,* 2013). In our study, out of 122 Gram Negative bacilli strains 31 (25.41%) carbapenem resistant were prevalent. And out of 31 carbapenem resistant isolates DDST detected higher number of MBL producers 29 (93.55%) than CDST 27 (87%). In Figure 1 *in vitro* antibiotic susceptibility testing showing organism resistant to 10µg Meropenem (A), combined disc synergy test showing ≥7mm increased size of zone of inhibition in Meropenem with EDTA combined disc (B), Double disc synergy test showing enhancement of zone of inhibition between Meropenem and EDTA disc (C) and blank disc showing no zone of inhibition for microorganism which was used as a control (D).

In conclusion, metallo-beta lactamases producing GNB isolates disseminated worldwide. So study finds that antibiotic surveillance should be at regular interval in hospital settings. And strict Antibiotic policy enforcing judicious use of antibiotics in the different clinical departments for effective control of carbapenem resistant bacteria either patient stay is longer. There is a importance to introduce a simple, cheap, reliable and reproducible screening tests for early detection and identification of MBL-producing GNB in routine diagnostics laboratories. So we advise that in diagnostic

procedure use additional EDTA disc (750µgm/ml) on routine AST plates and also screen by Meropenem- DDST method for MBL producers.

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