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Carbapenamase Detection: An Imperative Call

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Abstract: ***Aim: This study intended to determine carbapenamase production among Multi drug resistant gram negative bacteria by Disk Diffusion method, Vitek 2 and Modified Hodge and to determine MBL positive isolates by combined disk test and Double Disk Synergy Test. Methodology: In the present study , 550 gram negative isolates were isolated from different clinical samples and identified by Vitek 2 (BioMérieux). Antimicrobial susceptibility testing was performed by modified Kirby Bauer disk diffusion method using various antibiotics.MIC concentration was further determined by Vitek 2 .Carbapenamase production was identified by Modified Hodge Test and MBL positive isolates among carbapenem resistant isolates were determined by Combined Disk Method and Double Disk Synergy test. Results: Out of 550 isolates, 163(29.64%) exhibited resistance to carbapenem by disc diffusion and Vitek 2. Maximum number of carbapenem resistant isolate was obtained from urine (n=74), followed by Endotracheal secretion (n=27) and sputum (n=27). MHT for carbapenamase production was positive in 160 (98.2%) of the isolates. Carbapenamase production by MHT was highest with Acinetobacter spp. , n=15(100%) , Klebsiella spp. ,n=73(98.64%) , followed by E. coli spp. with n = 33 (97.1%) . MBL isolates, determined by combined disk and double disk synergy test were positive in 62(38 %) of the isolates and three isolates were negative for both MHT and MBL screening. Conclusion: Use of MHT, combined disc test and double disk synergy test with EDTA as a screening method can increase the sensitivity of detection of carbapenamase and can aid timely intervention to initiate infection control practices and thereby improve patient outcome.***

Keywords: Enterobacteriaceae, E test, Kirby Bauer disk, Modified Hodge test

1. Introduction

Gram negative enteric bacteria such as E. *coli*, *K. pneumoniae*, *Pseudomonas*, *Proteus* are common causes of hospital and community acquired infections that include cystitis, wound sepsis, pneumonia, peritonitis, bacteremia, and meningitis [1]. The main stay of treatment of these infections is by use of antibiotics, mainly beta lactam agents, which are the most commonly administered drug in the resource poor settings [2] . Excessive use of the beta lactams in treating infections caused by the multi drug resistant gram negative bacilli has stipulated strongly for the resistance exhibited by this group of organisms in recent years, the major mechanism being beta lactamase production[3,4]. Carbapenems have become the drug of choice to treat such infections. The WHO deems 'alarming levels' of antimicrobial resistance in commonly isolated bacteria in many parts of the world [5]. Perhaps most concerning is resistance to carbapenems which results from one or more of several different mechanisms: (hyper) production of ESBL or Ambler class C b-lactamases (including chromosomal AmpC b-lactamases) with concomitant loss of outer membrane porins; augmented drug efflux; alteration in penicillin-binding proteins; and carbapenemase production [6]. The expression of carbapenamase by Gram-negative organisms is of particular concern among these mechanisms. Klebsiella pneumoniae carbapenemase has been responsible for a number of outbreaks in the healthcare setting [7-10] and is one of the most frequently isolated Carbapenemases globally. Another carbapenemase, New Delhi metallo beta lactamase, was first identified in 2008 and has been already interpreted as one of the biggest antimicrobial resistance threats because it can be expressed by numerous pathogens, including Escherichia coli ST131, the strain associated with the global spread of CTX-M-15 ESBLs [10.] Moreover, these surveillance systems must be able to rapidly identify new threats and changing patterns in resistance [11]. Although the toolkits are concentrated on *Enterobacteriaceae*, it is recognized that carbapenem resistance is also of concern in other Gram-negative organisms, including *Pseudomonas* spp. and *Acinetobacter* spp [12] .Carbapenamase productions in gram negative bacteria may be detected phenotypically or by molecular methods. Based on this background, the aim of this study was to assess the prevalence of carbapenemase production among clinical isolates of multi drug resistant gram negative bacteria in a tertiary hospital in Faridabad and to detect Metallo Beta Lactamase in the area of study.

2. Materials and Methods

2.1 The Bacterial Isolates

A prospective study was orchestrated in a 350 bedded tertiary care centre in Faridabad, Haryana from October 2015 to April 2016. A total of 550 gram negative bacterial *isolates* were recovered from clinical specimens from different OPD and IPD patients (one isolate per patient). Collection of sample was done using strict aseptic precautions and was immediately processed. The isolates were obtained from various clinical specimens such as blood, pus, urine, lower respiratory secretions (endotracheal secretions, bronchoalveolar lavage) and sputum.

2.2 Identification of the isolates:

Identification of the isolates was done according to the conventional microbiological standard tests (Gram's stain, glucose fermentation test and oxidase test). Isolates identified as gram negative bacilli, glucose fermenters and oxidase negative were considered *Enterobacteriaceae*. The organisms were identified up to the species level using VITEK-GNI cards (bioMérieux, Marcy l'Etoile, France).

2.3. Antimicrobial Susceptibility Testing.

The antimicrobial susceptibility was performed by the Kirby Bauer's disc diffusion technique on Mueller-Hinton agar, as per Clinical Laboratory Standard Institute (CLSI) guidelines [13]. The antibiotics tested were as follows (potency in µg/disc) : Ampicillin(10µg) , Pipercillin/Tazobactam(10µg), Cefuroxime(30µg), Ceftriaxone (30µg), Cefoperazone/ Sulbactam (75µg), Cefepime (30µg), Amikacin(30µg), Gentamicin (10µg), NalidixicAcid (30µg), Ciprofloxacin (1µg), Nitrofurantoin (50µg), Colistin(10µg), Trimethoprim/ Sulfamethoxazole (1.2µg), Ticarcillin/ ClavulanicAcid (75µg), Ceftazidime (10µg), Cefepime, Aztreonam (30µg), Levofloxacin(5µg), Minocycline(10µg), Tigecycline(15µg) and carbapenems ; Ertapenem (10µg), Imipenem(10µg), Meropenem (10µg). *P. aeruginosa* ATCC *27853*, *E. coli* ATCC *25922*, *E. coli* ATCC *35218*, and *K. pneumoniae* ATCC *700603* were used as quality control strains. Those which were resistant to three or more classes of antibiotics were designated as MDR and were further evaluated for carbapenem resistance.

2.4. MIC Determination

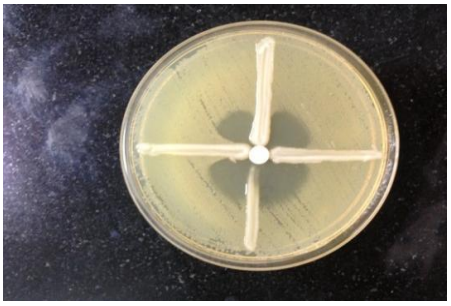
Minimum inhibitory concentrations (MICs) of antibiotics were determined by VITEK-2 AST-GN25 and AST-GN280 susceptibility cards in accordance with CLSI recommendations and manufacturers' instructions .

2.5. Phenotypic Screening for the Carbapenemase Production

Isolates with reduced susceptibility to meropenem and imipenem (diameter of zones of inhibition ≤13 mm) by disc diffusion method were screened for the production of carbapenemase. The phenotypic detection of the carbapenemase production was performed by the following methods.

3. Modified Hodge test

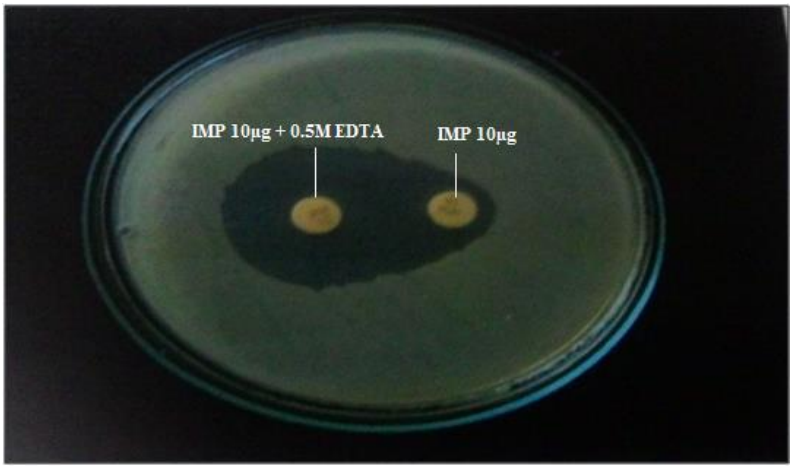
This test was performed by using meropenem discs (10 µg) as per CLSI guidelines[13]. A 0.5 McFarland standard suspension of *E. coli ATCC25922* was prepared in broth. A Mueller Hinton agar plate was inoculated as f or the routine disk-diffusion procedure. The plate was allowed to dry for 10 minutes. Meropenem disk was placed in the center of the plate. Using a 10 µl loop, three to five colonies of test organism grown overnight on a blood agar plate were picked and inoculated in a straight line out from the edge of the disk. The streak was at least 20-25 mm in length. Following incubation, Mueller Hinton agar was examined for enhanced growth around the test streak at the intersection of the streak and the zone of inhibition. For MHT *K. pneumoniae* ATCC BAA-1705 and BAA-1706 were used as positive and negative controls, respectively. The isolates which showed enhanced growth were considered positive for Modified Hodge test.



Modified Hodge Test positive clover leaf like pattern

Detection of Metallo Beta Lactamase

Metallo-beta-lactamase production was detected by double-disc synergy tests (DDST) with imipenem disc (10 ug) plus disc containing (750 µg) of EDTA as described earlier by Lee et al.[14]. Test organism was inoculated on to Mueller Hinton agar plates as recommended by the CLSI. An Imipenem (10 µg) disc was placed 20mm centre to centre from a disc containing 10µg Imipenem plus 0.5 M EDTA. After overnight incubation, a zone diameter difference of ≥ 7 mm between Imipenem disc & Imipenem plus EDTA disc were interpreted as Metallo-β-Lactamase positive. *K. pneumoniae* ATCC BAA-2146 and *P. aeruginosa* ATCC 27853 were used as positive and negative controls, respectively. Ratio of MICs of imipenem (IP) to IP plus EDTA(IPI)was carried out using MBL(IP/IPI) *E*-test method as per manufacturer's instructions.



Detection of MBL in *E.coli*

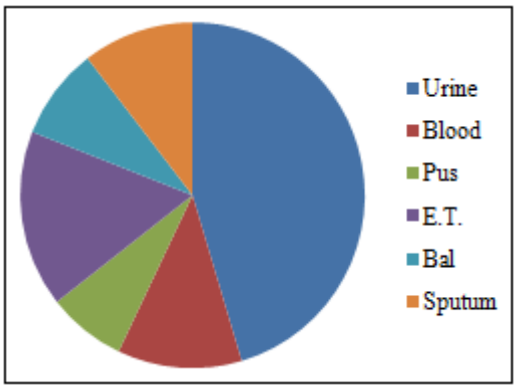
Combined Disk Test

Two Imipenem discs (10 µg), one containing 10 µL of 0.5 M (292 µg) anhydrous ethylenediaminetetraacetic acid (EDTA; Sigma Chemicals, St. Louis, MO, USA), were placed 25 mm apart (centre-centre). An increase in zone diameter of 7 mm or more around the Imipenem-EDTA disc compared to that of the Imipenem disc alone was considered positive for an MBL.

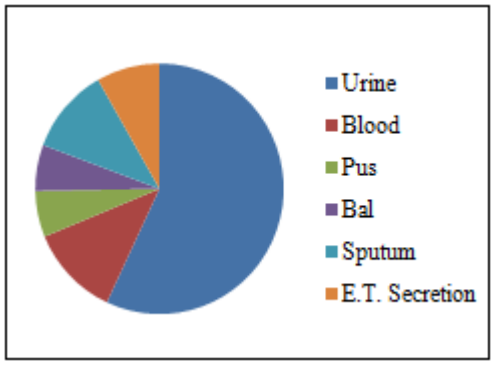
Detection of Carbapenamase production

A 550 non repetitive Gram negative bacteria (Enterobacteriaceae ,P. aeruginosa and A. baumanni) were isolated from 604 patients attending OPD and admitted in the various wards of hospital. The isolates were obtained from different clinical specimens such as urine (*n* = 74) , blood (n =19) , pus aspirates (*n* = 12) , Endotracheal secretion (n = 27) , sputum (*n* = 17) and Bronchoalveolar Fluids (n = 14) . Of the 550 isolates, 81% were Enterobacteriaceae , 9.82% were P. aeruginosa , and 9.2% were A. baumanni.

Carbapenamase production was detected in 29.64% of the isolates. Among the 163 strains, 95.7% (N=160) were detected by MHT and 36.2 %(N=62) by MBL screen. Both MBL and MHT screen were positive in 34 (19.6%) isolates. Three isolates tested negative by both the methods inspite of being resistant to carbapenem by disk diffusion. This might be due to the over production of ESBL, or Amp C hyper producers with porin loss.



Distribution of Carbapenenem Resistant Gram Negative bacilli samples used in the study(N =163)



Distribution of various clinical samples used in the study (N=550)

Sample	No. of CRE isolates	MBL producers
Urine	74	43(58.11%)
Blood	19	8(42.11%)
Sputum	17	3 (17.65%)
Pus	12	2 (16.67%)
Bal	14	1 (7.14%)
E.T. Secretion	27	6 (22.22%)

Distribution of MBL producers in various CRE clinical specimens

Organism	MHT Positive	MBL Positive	Both MHT+MBL Positive
E. coli (N =34)	33	12	6
Klebsiella Pneumoniae (N =74)	73	32	19
Enterobacter cloacae (N= 1)	1	1	1
Enterobacter Aerogenes (N= 1)	1	1	1
Providencia rettgeri (N=1)	1	0	0
Serratia Liquefaciens (N=1)	1	1	1
Proteus Mirabilis (N=5)	5	0	0
Serratia fonticola (N=3)	3	1	0
Acinetobacter baumanni (N=15)	15	11	4
Morganella Morgani (N=1)	1	0	0
Serratia Marcescens (N=1)	1	1	0
Burkholderia Cepacia(N=3)	2	0	0
Myorides (N=2)	2	0	0
Serratia ficaria (N=1)	0	0	0
Alcaligenes faecalis (N=1)	1	0	0
Pseudomonas Aeruginosa (N=16)	13	2	2
TOTAL	160	62	34

Detection of Carbapenamase production by MHT, Combined Disk Test and Double Disc Synergy Test .

4. Discussions

The present study was aimed at identifying carbapenem resistance in Gram-negative bacterial isolates from clinical samples received at the hospital laboratory. We were able to isolate carbapenem-resistant isolates from patients admitted in different units of the hospital, showing the widespread distribution of carbapenem resistance among our hospitalized patients. Our study was done only on MDR Gram-negative isolates. The prevalence may be different if all Gram-negative bacteria including the sensitive ones were assessed. MDR isolates were selected since identification of carbapenem resistance among them would be more significant clinically both for treatment purposes and for infection control.

Authors from different parts of India have recorded varying rates of carbapenem resistance in *Enterobacteriaceae* ranging from 5.75% to 51% in various gram negative bacilli over a decade. (Sathya Pandurangan et al., 2015 ;Atul Khajuria et al., 2014 ; Mita D. Wadekar et al., 2013; Rai et al ., 2014). This study was meant as a pilot study to identify the presence of Carbapenemases in our centre.

5. Conclusions

The present study reveals the emergence of carbapenem resistance among patients in a tertiary care hospital in Faridabad. The diverse mechanisms of carbapenem resistance, identified among these isolates, are a major cause for concern. Interventions to reduce the spread and burden of carbapenem-resistant Gram-negatives should take into account the role of international travel in the dissemination of these strains. Similarly, the role of hospital transfer in the introduction of these strains to healthcare facilities should be recognized. The introduction of targeted admission screening for carbapenem-resistant Gram-negative pathogens in high risk patients coupled with strict and effective infection control strategies may help reduce transmission and infection with these organisms in hospital. The increasing resistance in Gram-negative bacteria has been associated with heavy antibiotic use, including carbapenems [15].Thus, effective antibiotic stewardship in both clinical and community settings are important to reduce selective pressure, and to slow down or prevent the proliferation of resistant strains.

6. Acknowledgement

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