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RESEARCH ARTICLE

DETECTION OF CARBAPENEMASE PRODUCING ENTEROBACTERIACEAE BY MODIFIED HODGE TEST

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ABSTRACT

Introduction: Increasing rates of antimicrobial resistance have become a worldwide problem predominantly caused by Gram-negative bacteria, especially by the family of the Enterobacteriaceae. As a result, more patients need antimicrobial treatment using so called 'last resort' agents "The Carbapenems." This has resulted widespread carbapenem resistant mainly due to Carbapenemase enzymes. Their identification is of primary importance since carbapenemase producers are resistant not only to most (if not all) β-lactams but also to other main classes of antibiotics. The Modified Hodge Test (MHT), is a CLSI recommended, phenotypic test for detection of carbapenemase activity.
Aims and Objectives: The present study was undertaken to determine Carbapenemase resistance in Enterobacteriaceae from various clinical samples by Modified Hodge Test, a phenotypic method.
Materials and Methods: The present study was conducted in Department of Microbiology, Government Medical College and Associated group of hospitals, Kota (Raj.). One hundred consecutive, nonrepetitive Enterobacteriaceae isolates were processed for the study during the period of one year from September 2014 to August 2015. Antibiotic susceptibility test was performed by Kirby baeur method according to CLSI guidelines and the meropenem resistant isolates were further tested for Carbapenemase production by Modified Hodge Test (MHT).
Observation and Results: Among 100 enterobacteriaceae isolates 24 showed reduced susceptibility (intermediate or resistant) to Carbapenem. Carbapenem resistance was highest in *klebsiella spp*. (46.7%) followed by *Enterobacter spp*. (25%). and *E. coli* (16.1%).None of *proteus spp. and citrobacter spp* were carbapenem resistant. Modified hodge test was done on carbapenem non susceptible *Enterobacteriaceae* isolates, which detect Carbapenemase production in 18 (75%) of carbapenem resistant isolates.
Conclusion: To conclude, Carbapenemase producing Enterobacteriaceae isolates were relatively high in our institution. Accurate and timely detection of carbapenemase has important implications for efficient infection control and help in reducing the emergence of resistance thus decreases the morbidity and mortality rate.

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INTRODUCTION

Increasing rates of antimicrobial resistance have become a worldwide problem predominantly caused by Gram-negative bacteria, especially by the family of the Enterobacteriaceae. Enterobacteriaceae family are important causes of urinary tract infections (UTIs), various intra- abdominal infections, bloodstream infections, hospital and healthcare associated pneumonias. Enterobacteriaceae spread easily between humans by hand carriage as well as contaminated food and water as they are normal inhabitants of the intestinal flora and have a propensity to acquire genetic material through horizontal gene transfer, mediated mostly by plasmids and transposons. (Partridge, 2011; Stokes and Gillings, 2011) Third-generation cephalosporins were originally developed as β-lactams able to overcome resistance caused by common lactamases. However, within a few years, hospital-acquired gram-negative bacilli like Klebsiella pneumoniae and others began producing mutated versions of these β-lactamases that made them resistant to third-generation cephalosporins and to the monobactam. (Paterson, 2006) Since 2000s, the spread of community acquired E. coli isolates producing extended spectrum β-lactamases (ESBLs) capable of hydrolyzing almost all β-lactam antibiotic except carbapenems has been reported worldwide. (Pitout and Laupland, 2008) As a result, more patients need antimicrobial treatment using so called 'last resort' agents "The Carbapenems." (Paterson, 2006; Paterson

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et al., 2001) This has resulted in an increase in the antibiotic pressure on carbapenems which lends to the activation of resistance genes against these drugs. (Paterson *et al*., 2001) Resistance to carbapenem may be due to (1) production of β-lactamases (carbapenemases) that hydrolyse the carbapenems, (2) changes in outer-membrane porins that block the entry of these antibiotics, (3) active pumping of the antibiotic out of the cell using complex "efflux pumps." Strains that do not produce carbapenemase but which are carbapenem resistant due to other mechanisms are usually less resistant to antibiotics of other families. Their carbapenem resistance trait is not transferable, as opposed to most of the strains harboring carbapenemase genes. For this reason, carbapenem resistant isolates that do not produce carbapenemases are considered of less clinical concern than carbapenemase producing strains. (Nordmann *et al*., 2012)

Carbapenemase mediated carbapenem resistance in Enterobacteriaceae The first carbapenemases identified in Enterobacteriaceae were SME-1 in London in 1982 (Yang, 1990) and IMI-1 in the USA in 1984. (Rasmussen *et al*., 1996) Since then, carbapenem resistant Enterobacteriaceae have been reported worldwide, primarily as a consequence of widespread acquisition of carbapenemase genes. (Queenan and Bush, 2007) Their identification is of primary importance since carbapenemase producers are resistant not only to most (if not all) β-lactams but also to other main classes of antibiotics.

Detection and surveillance of carbapenemase-producing organisms is important for the selection of appropriate therapeutic schemes and the implementation of infection prevention measures.Carbapenemase gene detection by molecular methods is the gold standard but is available in only a few reference laboratories, so there is a need for simple and reliable phenotypic test. The Modified Hodge Test (MHT), is a CLSI recommended, phenotypic test for detection of carbapenemase activity. (Clinical Laboratory Standards Institute, 2011) It is based on the inactivation of carbapenem by carbapenemase producing strains that enables a carbapenem susceptible indicator strain to extend growth toward a carbapenem containing disk, along the streak of inoculum of the tested strain. (Girlich *et al*., 2012) The Carbapenamaseproducing Enterobacteriaceae are associated with high rates of morbidity and mortality particularly amongst critically ill patients. Antimicrobial treatment options for these multidrug resistant infections are limited. Only a few antimicrobial agents (e.g. colistin, tigecycline, fosfomycin and amikacin) with an uncertain in vivo efficacy and/or reported toxicity are left to treat these infections.

Aims and Objectives

The present study was undertaken to determine Carbapenemase resistance in Enterobacteriaceae from various clinical samples by Modified Hodge Test, a phenotypic method.

MATERIALS AND METHODS

The present study was conducted in Department of Microbiology, Government Medical College and Associated group of hospitals, Kota (Raj.). Various clinical samples were obtained from the patients who came in various outdoor and indoor departments of M.B.S. Hospital and N.M.C Hospital, Kota. One hundred consecutive, nonrepetitive Enterobacteriaceae isolates were processed for the study during the period of one year from September 2014 to August 2015. Clinical samples mainly included Blood, Urine, sputum, Surgical-site infections, other woundsand Throat swab. Samples were processed within two hours of receipt as per standard procedures. (Gross and Holmfs, 2006) All Enterobacteriaceae isolates were identified by conventional methods. The organisms were identified upto species level based on colony morphology, Gram stain, motility and by standard biochemical tests which include catalase, oxidase, indole, methyl red, vogesproskaeur, citrate, urease, TSI, Phenylalanine deaminase test, Aminoaciddecaroxylation test, Sugar Fermentation test (glucose, sucrose, maltose, lactose). (Forbes *et al*., 1998) Antibiotic susceptibility test was performed by Kirby baeur method according to CLSI guidelines and the meropenem resistant isolates were further tested for Carbapenemase production by Modified Hodge Test (MHT). (Clinical Laboratory Standards Institute, 2011)

Detection of Carbapenemase production by MHT

Escherichia coli ATCC 25922 was cultured overnight and suspended to achieve a 0.5 McFarland standard turbidity and was lawn cultured onto a MHA plate using a sterile cotton swab. After drying, the disc containing Meropenem (10μg) was placed at the center of the plate, and an overnight cultured test strain was heavily streaked from the center to the periphery of the plate. The presence of a distorted zone after overnight incubation was interpreted as a positive result. (Clinical Laboratory Standards Institute, 2011) MHT Positive *Klebsiella pneumoniae* ATCC1705 and MHT Negative *Klebsiella pneumoniae* ATCC1706 were used for quality control.

OBSERVATION AND RESULTS

The present study was carried out in the department of Microbiology, Government medical college, Kota. A total of 100 Enterobacteriaceae isolates were studied. In this study

majority of the *Enterobacteriaceae*were isolated from urine samples 63%, followed by pus 21%, sputum 11% and blood 5% respectively (Table 1). The Enterobacteriaceae isolated were: mainly *E.coli*(55%), followed by *Klebsiella* spp. (30%), *Citrobacter* spp. (6%), *Proteus* spp. (4%), *Enterobacter* spp. (4%) (Table 2). Among 100 enterobacteriaceae isolates 24 showed reduced susceptibility (intermediate or resistant) to Carbapenem (Table 3). Carbapenem resistance was highest in *klebsiella spp*. (46.7%) followed by *Enterobacter spp*. (25%). and *E. coli* (16.1%). None of *proteus spp. and citrobacterspp* were carbapenem resistant (Table 4). Modified hodge test was done on carbapenem non susceptible *Enterobacteriaceae* isolates, which detect Carbapenemase production in 18 (75%) of carbapenem resistant isolates (Table 5). Carbapenemase production was detected by MHT in 100% of *Enterobacter spp*., 78.6 % of *Klebsiella spp.* and 66.7% of *E.coli.* However, carbapenemase production could not be detected using MHT in 33.3% and 21.4% of *E. coli* and *Klebsiella spp.* respectively (Table 6). This negative result could be due to other important causes of carbapenem resistance among *Enterobacteriaceae* such as overproduction of ESBL or AmpC enzyme with porin loss. (Nordmann *et al*., 2012)

Table 1. Sample wise Distribution of study subjects (n=100)

Sample	Number (%)	Percentage
URINE	63	(63%)
PUS	21	(21%)
SPUTUM	11	(11%)
BLOOD	5	(05%)
Total	100	(100%)

Table 2. Distribution of *Enterobacteriaceae* isolates among study subjects (n=100)

Organism	Number	Percentage
E.coli	56	(56%)
Klebsiella	30	(30%)
Citrobacter	6	(6%)
Enterobacter	4	(4%)
Proteus	4	(4%)
Grand Total	100	(100%)

Table 3. Distribution of carbapenem resistant and carbapenem sensitive isolates of *Enterobacteriaceae* (n = 100)

Carbapenem	Number	Percentage
Resistant	24	24%
Sensitive	76	76%
Total	100	100%

Table 4. Distribution of carbapenem resistant *Enterobacteriaceae* (n=24)

Enterobacteriaceae isolates	Total	Carbapenem Resistant (n=24)	
		Number	Percentage
Citrobacter	6	0	0
E.coli	56	9	16.1
Enterobacter	4	1	25
Klebsiella	30	14	46.7
Proteus	4	0	0
Total	100	24	100

Table 5. Distribution of carbapenamase producer and non-producer in *Enterobacteriaceae* family based on Modified hodge test (n = 24)

MHT	Number	Percentage
NEGATIVE	6	25
POSITIVE	18	75
Total	24	100

Table 6. Organism wise distribution of carbapenamase producer and non-producer in *Enterobacteriaceae* family based on Modified hodge test (n = 24)

Isolates	MHT Positive	MHT Negative	Total
E.coli	6 (66.7)	3 (33.3)	9 (100)
Klebsiella	11 (78.6)	3 (21.4)	14 (100)
Enterobacter	1 (100)	0	1 (100)
Grand Total	18 (75)	6 (25)	24 (100)

DISCUSSION

Carbapenems are considered as the best treatment options for *Enterobacteriaceae*. The emergence and proliferation of bacteria resistant to this important group of drug are jeopardizing the use of carbapenems. (Datta *et al*., 2012) Molecular methods are currently the gold standard for detection of carbapenemases, however, these methods cannot be performed routinely in the clinical microbiology laboratory. Therefore, rapid and easy identification and presumptive characterization of Carbapenemase producing Enterobacteriaceae are required. (Saito *et al*., 2014) In the present study majority of the *Enterobacteriaceae*were isolated from urine samples (63%) (Table 1). Study done by Julie A Creighton *et al*. (Creighton, 2014) and Gautam (Gautam and Lekhak, 2015) reported similar results with majority of *Enterobacteriaceae* isolates from urine, (80.7%) and 76.5% respectively. This is obvious as *Enterobacteriaceae* may account for 80 percent of clinically significantisolates of gram-negative bacilli in clinical microbiology laboratories. They account for more than 70 percent of urinary tract infections.But present study does not correlate with the study done by Mulla *et al*. (2011), who repoertd 30.9 % urinary isolates but in their study maximum number of isolates were from urine samples, which again correlate with the present study. In the present study the most frequently isolated *Enterobacteriaceae* member was *E.coli* (55%) and *Klebsiella* spp. (30%) (Table 2) which is similar to study done by Mulla *et al*. (2011) who reported *E.coli*(55.6%) and *Klebsiella* spp. (31.2%) and Po-Yu Liu *et al*. (2014) who reported *E.coli*(43.7%) and *Klebsiella* spp. (25.8%) as major pathogen among *Enterobacteriaceae.*In the present study *Citrobacter* spp. was isolated from (6%) samples which is similar to Po-Yu Liu *et al*. (2014) (5.7%), and *Proteus* spp. was isolated from (4%) samples which is similar to Mulla *et al*. (2011) (3.3%).

In the present study 24% (Table 3) *Enterobacteriaceae* isolates were carbapenem resistant which is similar to study done by Mulla *et al*. (2011) (30.2%). Present study does not correlate with the study done by Trupthigowda *et al*. (2015) and Datta *et al*. (2012) who reported 14%, 7.87% lower resistant respectively and pandurangan *et al* who reported 57% carbapenemase resistant which is higher as compare to present

study. Datta *et al* has Antibiotics Stewardship Programme in their hospital, which may be a contributing factor for low prevalence of CRE in their study. Siegel *et al*. (2006) Showed antimicrobial stewardship to be an important part of efforts to control multidrug resistant organisms. In the present study Carbapenem resistance was highest *in Klebsiella spp*. (46.7%) followed by *Enterobacter spp*. (25%). and *E. coli* (16.1%). (Table 4)Similar to this study, a high prevalence of resistance to carbapenems 2-13% in *E. coli* and 31-51% in *Klebsiella spp*. has been reported by Wattal *et al*. (2010) from Delhi. Similarly, a high prevalence of resistance to carbapenems 14.64% in *E. coli* and 29.69% in *Klebsiella spp*. has been reported from uttarpradesh by Chauhan *et al*. (2015) Present study does not correlate with study done by Trupthi *et al*. and Gautham *et al*. who reported *E. coli* as highest carbapenem resistant. This variation in antibiotic susceptibility could be due to geographical difference, pattern of antibiotic use and selective pressure exerted by antimicrobial drugs on bacteria. In the present study carbapenemase production by MHT was detected in 75% isolates (Table 5), which is comparable to study done by Amjad *et al*. (2011) who reported 69% sensitivity of MHT and higher as compare to study done by S. pandurangan *et al*. (2015) and TrupthiGowda *et al*. (2015) who reported 53.5% and 62.1% MHT positive among carbapenem resistant *Enterobacteriaceae.* This study does not correlates with the study done by Lavinia N. Arend *et al*. (2015) who reported 83.2% isolates positive by MHT which is higher as compared to present study.

This may be due to the fact that Lavinia N. Arend *et al* studied MHT test on KPC 2 producing isolates and CLSI has reported that MHT has a very high sensitivity and specificity in detection of KPC carbapenamases. Carbapenemase production was more in *Klebsiella spp.* compared to *E. coli* in the present study (Table 06). Similar findings have been reported in studies done by Datta *et al*. (2012) in 2012 and Gupta *et al*. (2006) in 2006 from North India. The emergence and proliferation of Carbapenemase producing Enterobacteriaceae should alert that all isolates showing intermediate or resistant zone diameter on disc diffusion testing should be further tested for production of carbapenemases by Modified Hodge test to avoid treatment failures and development of resistance due to unnecessary use of this class of antibiotic. (Datta *et al*., 2012)

Conclusion

To conclude, Carbapenemase producing Enterobacteriaceae isolates were relatively high in our institution. The significant finding of our study was that 75% of Enterobacteriaceae isolates which showed non susceptible zone sizes for Carbapenem on disc diffusion test were detected positive by MHT. Accurate and timely detection of carbapenemase has important implications for efficient infection control and help in reducing the emergence of resistance thus decreases the morbidity and mortality rate.

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No conflicts of interest present.

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