Detection of Carbapenemase Producing Enterobacteriaceae by Modified Hodge Test in Rajshahi Medical College Hospital

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Keywords:
Carbapenemase, Enterobacteriaceae, Modified Hodge Test, Carbapenem resistant.

Abstract

Background: *Carbapenem drugs are used as last resort antibiotic for the treatment of extended spectrum beta-lactamase (ESBL) and AmpC beta lactamase enzyme producing gram negative bacteria. But some bacteria produce carbapenemase enzymes and hydrolyze these drugs. Carbapenamase genes are present on plasmid and these plasmid can contain other drug resistant gene also as well as these plasmid can transfer to other bacteria of the same or different species. So extended drug resistant bacteria originate and disseminate. Misuse of antibiotics have a role for these purposes which cause gene mutation and appearance of new mechanism of drug resistant. Members of enterobacteriaceae are gram negative bacteria and many of them are normal flora of human colon and can easily be spread among human. So, carbapenemase producing enterobacteriaceae can disseminate in the hospital and make infection control difficult. There are a few antibiotics remaining for the control of infection with such type of bacteria. So, early detection of these enterobacteriaceae and rational use of antibiotics are essential for infection control in hospital.*

Aims and objectives: *The aim of this study is to identify carbapenemase producing enterobacteriaceae which would help proper infection control.*

Materials and methods: *A descriptive type of study was carried out for the detection of carbapenemase producing enterobacteriaceae members in the department of Microbiology, department of Surgery and its allied branches of Rajshahi Medical College and Hospital. A total 233 enterobacteriaceae were isolated from wound swab and antibiogram were done using standard procedure between January 2014 to Decmber 2014. The enterobacteriaceae isolates which showed resistant to both meropenem (Zone of inhibition d" 22mm) and ceftriaxone were studied for the detection of carbapenemase production by Modified Hodge test(MHT).*

Results: *Within 152 meropenem and ceftriaxone resistant isolates 47 (30.92%) showed carbapenemase production by MHT. The species distribution amongcarbapenemase producer were*Escherichia coli*68.08%,* Proteus *spp. 12.77%,* Enterobacter *app. 17.02%,* Klebsiella *app. 02.12% and* Providentia *spp. 0.00%*

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Introduction:

Carbapenemase enzymes producing gram negative bacterial infection is an everyday's challenge for the clinicians to combat with. In the last two decades, gram negative bacteria gain more importance than gram positive bacteria regarding this problem.[1,2] The prevalence of carbapenemase producing bacteria in France is 3%- 5% and in India more than 80%.[3]

Clinicians often use beta-lactum drugs like penicillin, cephalosporin, monobactum etc for infection control. But extended spectrum beta-lactamase (ESBL) and AmpC beta-lactamase producing gram negative bacteria are resistant to those antibiotics in most cases now. Thus, carbapenem group of beta-lactum drugs i.e imipenem, meropenem, doripenem, itrapenem etc are now used as last resort antibiotics for controlling gram negative becterial infection.[4] But many members of gram negative bacteria again becomes resistant to carbapenem antibiotics by producing carbapenem hydrolyzing carbapenemase enzymes besides

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modification of membrane permeability, excessive drug efflux or by producing certain ESBL or AmpC beta-lactamase with increase carbapenem hydrolyzing capacity. These occur most often due to gene mutation by misuse of antibiotics.[5]

There are three classes of carbapenemase enzymes belonging to Ambler classification of beta-lactamase enzymes such as class A, class B and class D.[6] Class A carbapenamase include *Klebsiella pneumoniae* carbapenemase (KPC), *Serratia marcescens* enzyme (SME), in Imipenem hydrolyzing enzyme (IMI) etc. KPC are the most common and found mostly from *Klebsiella pneumoniae*and to a lesser extent from Escherichia coli and other enterobacteriaceae species. Class B carbapenemase include Active on imipenem (IMP) carbapenemase, Verona-Integron encoded beta-lactamase (VIM), New Delhi metallobeta-lactamase (NDM) etc. These enzymes mainly produced by *Klebdiella pneumoniae*, *Escherichia coli (E. coli)*, *Serratia* spp. and other enterobecteriaceae species. Oxa-48, Oxa-181 etc are class D carbapenemase and produced mainly by *Klebsiella pneumoniae* & *Escherichiacoli*.[7] Death rate among KPC producing bacterial infection attributed to 50% and that of the MBL producers infection is 18%-64% whereas that in case of OXA-48 is unknown.[7-9]

Enterobacteriaceae is a group of gram negative becteria among others. Many members of enterobacteriaceae are normal flora of colon and cause various infection as wound infection, urinary tract infection, peritonitis, meningitis, pneumonia, septicaemia etc. They can spread easily among human by hand contact and by contaminated food and water. They carries the gene for carbapenemase enzymes mainly on their plasmid. So, they have the capacity to transmit it to other bacteria of same or different species by conjugation and contribute drug resistant transfer. These phenomenon occurs mainly in hospitals and its surrounding environment. The bacteria carries genes for carbapenemase enzymes may also carry other drug resistant gene in their plasmid resulting in extended drug resistant (XDR) highly virulent bacteria. There are a few antibiotics against such type of bacteria.[6,10,11]

Bacteira producing carbapenemase enzyme can be detected by molecular methods and enzyme detection methods. Molecular methods are polymerase chain reaction (PCR), DNA sequencing, iso-electric focusing, spectrophotometry etc.[12] Enzyme detection methods include Modified Hodge test(MHT), double disc synergy test, combined disc test, EDTA-imipenem microdilution MIC test, E-test, MBL strip test etc. Molecular methods detect carbapenemase enzyme encoding gene but enzymatic methods detect carbapenemase enzyme activity. MHT is a CLSI recommended low cost enzyme detection method and it can easily be performed in every microbiology laboratory fascilities.[13]

Objectives:

The aim of the study was to identify carbapenemase producing enterobacteriaceae in Rajshahi Meidcal College Hospital. This would help in early detection of these resistant pathogen, early start of proper treatment and their effective control.

Materials and methods

Total 233 enterobacteriaceae were isolated from wound swab in microbiology laboratory in Rajshahi Medical College during the period of January, 2014 to December, 2014. Standard methods were employed for the collection of sample and isolation and identification of the organism. The identified enterobacteriaceae were studied for drug sensitivity by modified CarbyBauer disc diffusion method on Muller-Hinton agar media. Antibiotic disc were Meropenem (10 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Ceftriaxone (30 µg), Aztreonam (3 µg), Gentamycin (10 µg), Amicacin (30 µg), Azythromycin (15 µg), Collistin (10 µg) and Cloramphenicol (30 µg). Zone of inhibition was detected by CLSI, 2012 recommendation and technical data of Hi-media, 2012. The isolated enterobacteriaceae which showed zone of inhibition to meropenem d" 22 mm and resistant to cephtriaxone were considered as carbapenemase producing enterobacteriaceae. The carbapenemase producer were confirmed by MHT.[13,14]MHT can detect carbapenemase producers with sensitivity and specificity of 94% and 100% respectively.[15]

Procedure of Modified Hodge test: 5 ml inoculum of *E.coli* (ATCC 25922) was prepared and standardized by 0.5 McFarland standard. The inoculum was diluted 1:10 by adding 4.5 ml of sterile normal saline. The diluted inoculum was spread on Muller-Hinton agar plate with cotton swab and allowed to dry in air for 3-10 minutes then an Imipenem (10 µg) disc was placed at the centre of the plate. A straight line was drawn with the help of inoculating wire loop containing identified test bacteria from margin of the

disc to the end of the Muller-Hinton agar plate. 4 identified test bacteria were tested in a single Muller-Hinton plate and incubate overnight at 37p C. Reading was taken after 24 hrs to see clover-leaf type of indentation at the intersection of the test bacteria and the *E. coli* (ATCC 25922) within the zone of inhibition. Positive result was indicated by presence of clover-leaf like indentation of the *E.coli* (ATCC 25922) along the streak line of test bacteria within the zone of inhibition. Negative result showed no growth of *E. coli* (ATCC 25922) along the test bacterial streak within the zone of inhibition. Indeterminate result showed by inhibition of the growth of *E. coli* (ATCC 25922) around the streak line of test bacteria.[4,16]

Result:

A total 233 enterobecteriaceae was isolated among them *E. coli* 122 (52.35%), *Proteus* spp. 54 (23.17%), *Enterobecter* spp. 37 (15.88%), *Klebsiella* spp. 14 (6.00%) and *Providentia* spp. 6 (2.58%). Within those enterobacteriaceae both Meropenem and Ceftriaxone resistant were 152 (65.24%) where *E. coli* 70.49% (86/122), *Proteus* spp. 51.85% (28/54), *Enterobecter* spp. 72.97% (27/37), *Klebsiella* spp. 57.14% (8/14) and *Providentia* spp. 50.00% (3/6). Carbapenemase producer among these resistant isolates were 30.92% (47/152) and the species distribution among them were *E. coli* 37.21% (32/86), *Proteus* spp. 21.43% (6/28), *Enterobecter* spp. 29.63% (8/27), *Klebsiella* spp. 12.50 % (1/8) and *Providentia* spp. 0.00% (0/3). Distribution of carbapenemase producing enterobacteriaceae species within total carbapenemase producing enterobacteriaceae were *E. coli* 68.08% (32/47), *Proteus* spp. 12.77% (6/47), *Enterobacter* spp. 17.02% (8/47), *Klebsiella* spp. 2.12% (1/47) and *Providentia* spp. 0.00 % (0/47).

Table I: *Enterobacteriaceae species isolated from wound swab*

Organism isolated	Numbers	Percentage
E.coli	122	52.35%
Proteus spp.	54	23.17%
Enterobacter spp.	37	15.88%
Klebsiella spp.	14	6.00%
Provindentia spp.	6	2.58%
Total	233	100%

Table II : *Organism resistant to Meropenem and both Meropenem and Ceftriaxone:*

Organism	Meropenem resistant	Meropenem+Ceftriaxone resistant
E.coli (N=122)	88 (72.13%)	86 (70.49%)
Proteus spp.(N=54)	30 (55.55%)	28 (51.85%)
Enterobacter spp.(N=37)	27(72.97%)	27 (72.97%)
Klebsiella spp. (N=14)	8(57.14%)	8 (57.14%)
Provindentia spp.(N=06)	3(50%)	3 (50%)
Total (N=233)	156 (66.95%)	152 (65.24%)

N=Number

Table III : *Carbapenemase producing enterobacteriaceae isolates among Meropenem+ Ceftriaxone resistant isolates*

Number of resistant organisms	Number of Carbapenemase producing organisms	Percentage
E.coli (N=86)	32	37.21%
Proteus spp.(N=28)	06	21.48%
Enterobacter spp.(N=27)	08	29.63%
Klebsiella spp. (N=08)	01	12.50%
Provindentia spp.(N=03)	00	00.00%
Total (N=152)	47	30.92%

N=Number

Table IV: *Distribution of Carbapenemase producing enterobacteriaceae isolates among total carbapenemase producing enterobacteriaceae.*

Name of the organisms	Total number of Carbapenemase producers	Percentage
E.coli (N32)	47	68.08%
Proteus spp.(N=06)		12.77%
Enterobacter spp.(N=08)		17.02%
Klebsiella spp. (N=01)		02.12%
Provindentia spp.(N=00)		00.00%

N=Number

Figure 1: *Positive result shows by bacteria of 4R striking line in Modified Hodge test.*

Discussion:

Enterobacteriaceae are resistant to carbapenem drugs due to production of carbapenemase enzymes, increase membrane permeability, excessive drug efflux and by production of ESBL or AmpC beta-lactamase enzymes with increase carbapenem hydrolysing capacity. In our study isolated enteriobacteriaceae showed resistant to carbapenem drugs was 66.95%.In a similar study at Franch by Birgy A et al, 2012 [17]showed Meropenem resistant 53.33% which is nearly similar to our study. A study in Dhaka by Noorjahan Begum and S.M. Shamsuzzaman[18] and a study in Mumbi, India by Nair P.K. and Vaz M.S.[19]showed the prevalence of carbapenem resistant was 14.49% and 12.26% respectively which are much lower than our study. In another study by Priyadarshini Shanmugam et al, 2013in Chennai, India[20] showed 93.4% enterobacterioceae were resistant to Meropenem which is much higher than our study. The dissimilarities between different studies may be due to the random use of 3rd generation cephalosporins and carbapenem without culture and sensitivity which leads to the emergence of resistance to them and their dissemination throughout the hospital. This dissimilarities may also be due to inadequate measure taken to prevent the spread of resistant pathogen, no antibiotic policy in our hospital and inadequate antibiogram of imperical antibiotic therapy.

In our study, 30.92% of isolated enterobacteriaceae was detected carbapenemase producer by Modified Hodge test which is nearly similar to a study in Hyderabad, India where Ramana KV. et al, 2013[21] showed carbapenemase production in enterobacteriaceae was 35.9%. Our study is also similar to a study of Cury AP et al, 2012 in Brazil[22] and that showed carbapenemase production within carbapenem resistant isolates were 35.46% by Modified Hodge test. But our study is dissimilar with the study of Priyadarshini Shanmugam et al, 2013[20] and Arend et al, 2015[23] who reported 82.6% and 83.23% isolates were positive by MHT which are much higher in comparison to our study.

In our study carbapenemase production within carbapenem resistant isolates of same enterobacteriaceae species were *E.coli* 37.21%, *Proteus* spp. 21.43%, *Enterobacter* spp. 29.63%, *Klebsiella* spp. 12.50% and *Provindentia* spp. 00.00% which is dissimilar with the study of Priyadarshini Shanmugam et al, 2013 in Chennai, India[20] who found *E. coli* 80.95%, *Proteus* spp. 100%, *Klebsiella* spp. 86.36% and *Citrobacter* spp. 50% And with a study of Anita E. et al, 2016 in Rajasthan, India[24] who showed E.coli 66.7% *Klebsiella* spp.78.6 % and *Enterobacter*spp

100% where*Proteus* spp and *Citrobacter* app.were not resistant to carbapenem which are also dissimilar with our study. In our study the species distribution among the enterobacteriaceae isolates which produces carbapenemase by MHT were *E.coli* 68.08%, *Proteus* spp. 12.77%, *Enterobacter* spp.17.02% *Klebsiella* spp. 2.12% and *providentia* spp. 0.00%. In a similar study by Ramana KV. et al, 2013 from Hyderabad, India[21] showed *E.coli* 19.28%, *klebsiella* spp. 40.61%, *Proteus* spp. 4.57%, *Enterobacter* spp. 22.84% and *Citrbacter* spp. 12.69% and Priyadarshini Shanmugam et al, 2013 from Chennai India[20] showed *E.coli* 44.74% *Klebsiella* spp. 50%, *Proteus* spp. 2.63% and *Citribacter* spp. 2.63%. Those study are dissimilar to our study. In another study of Dr. Ph. Henkhoneng Mate et al, 2014 in Monipur, India[28] found *E.coli* was 88.89%, *Klebsiella* spp. 5.55%, *Proteus* spp. 5.55% which is dissimilar to our study. In a study, Bora et al, 2014 at Bharatpur, Nepal[16] found *E.coli* was 51.25% and *klebsiella* spp. 48.75% which is also dissimilar to our study. In the study of Datta et al, 2012 in Chandigarh[25] and Smita sood, 2014 in Joypur, India[26] showed carbapenemase producing *klebsiella-pneumoniae* was 0% and 100% respectively, which are also dissimilar with our study. In the study by Hayder et al, 2012 of Dhaka, Bangladesh[27] and a combind study in India, Pakistan and UK by Kumarassamy K K et al, 2010[1] showed carbapenemase producing *Klebsiellapneumoniae* were 4.8% and 1.7& respectively which are also dissimilar to our study. The dissimilarities may be due to the prevalence of carbapenamese producing gram-negative bacilli varies greatly from country to country and among different institution within the same country.[29] The dissimilarities may also be due to defective culture and sensitivity report and inadequate dose and duration of used antibiotics. Other factors may be organism varies in different geographical location and in different environment as well as sanitation habit of the patients and variation of antibiotic use in different hospital.

Though PCR is the gold standard for carbapenemase producing bacteria detection but we were unable to use that procedure due to lack of facilities. It is the limitation of the study.

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