Detection of metallo-beta-lactamase producing enterobateriaceae from wound infection in RajshahiMedical College Hospital

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Abstract:

Background: Wound infection is a common problem and a wide range of bacteria including enterobacteriaceae are responsible for it. Multidrug resistant enterobacteriaceae are greatest risk for the management of wound infection as they produce beta -lactamase enzymes which cleaves beta-lactam drugs.Metallo-beta lactamase (MBL) is a member of beta-lactamase enzymes which are produced by gene mutation due to misuse of antibiotics. **Objective**: Detection of metello-beta-lactamase producing enterobacteriaceae from wound infection. **Methods**: A descriptive type of study was carried out for the detection of MBL in the Departments of Microbiology and Surgery & its allied branches of Rajshahi Medical College and Hospital. A total 233 enterobacteriaceae were isolated and antibiogram were done from wound swabs . The enterobacteriaceae which showed resistant to both meropenem and ceftriaxone were used for the detection of MBL by double disk synergy test**. Results:** Among the enterobacteriaceae, *E.coli* 86(70.49%), *Proteus* spp. 28(51.85%), *Enterobacter* spp. 27(72.97%), *Klebsiella* 8 (57.14%) and *Providentia* spp. 3(50%), were resistant to both meropenem and ceftriaxone. Detected MBL were 66(76.74%), 19(67.85%), 21(77.77%), 7(87.50%) and 1(33.33%) from *E. coli*, *Proteus spp.*, *Enterobacter spp.*, *Klebsiella spp.* and *Provendentia spp.* respectively. **Conclusion:** Multidrug resistant enterobacteriaceae was due to production of MBL as result of mutation of genes for misuse of antibiotics without during antibiogram .

Key words: Metallo-beta-lactamase, Carbapenemase, multidrug resistant, wound Infection.

Introduction

Wound infection is a common problem in hospitals throughout the world and is caused mainly by bacteria. A wide range of bacteria including enterobacteriaceae are responsible for wound infections.Healing needs good healthy environment of the wound which can be provided with regular dressing and antibiotic therapy.[1] But bacteria can develop resistant against antibiotics by different mechanisms. One of the mechanisms is the production of beta-lactamase enzymes which hydrolyze the beta-lactam drugs like penicillins, cephalosporins, monobactum, carbapenems etc. These enzymes present in the periplasmic space of gram-negative bacteria and destroy the drugs before they bind with target structures. Enterobacteriaceae carry genes for beta-lactamase, an enzyme present in their chromosomes, plasmids and transposons.[2] Many newer beta-lactam drugs have been developed that act against beta-lactamase producing bacteria. But genes that code for beta-lactamase enzymes have mutated continuously in response to heavy use of antibiotic leading to the development of newer broad spectrum beta-lactamases.[3] Besides that intraspecies and interspecies transmission of mutant genes occur by conjugation which also contribute drug resistance.[2,4-6] These mutation mostly occur within the hospitals and surrounding environment.

Carbapenems, the newer class of beta-lactam drugs which include imipenem, meropenem, doripenem and ertapenem are stable and not destroyed by extended spectrum beta-lactamase and Amp C beta-lactamase.[7] These drugs are the choice for the management of serious hospital acquired infections caused by multidrug resistant enterobacteriaceae.[8,9] Unfortunately enterobacteriaceae again

develop resistant to carbapenems by producing metallo-beta-lactamase and other carbapenemase enzymes. In the recent year worldwide outbreak of carbapenem resistant enterobacteriaceae have been increasingly reported.[2,10,11] These enterobacteriaceae are also resistant to beta-lactamase inhibitors like clavulanic acid and tazobactam.[12,13]

Carbapenemase enzymes belong to 3 molecular classes, such as class A, B & D.[2] Class B carbapenemase enzymes use zinc at their active site and inhibited by EDTA (ethylene diamine tetra acetic acid), known as metallo-beta-lactamase (MBL). Class B carbapenemases are active on imipenem carbapenemase, Verona-Integron encoded metallo-beta-lactamase, Sao Paulo metallo-beta-lactamase, German imipenemase , Seoul imipenemase and New Delhi metallo-beta-lactamse(NDM). MBL enzymes hydrolyze all beta-lactam antibiotics and clavulanic acid except aztreonam.[14] These enzymes mainly present in *Klebsiella pneumoniae, Escherichia coli*, *Serratia* spp. and other enterobacteriaceae species.[2,15,16]

In a study at international center for diarrhoeal disease and recharch (ICDDRB), Dhaka, Bangladesh showed among 403 isolates, 3.5% were positive for MBL and predominant species were *Klebsiellapneumoniae, Acinetobacter* and *Escherichia coli.* [17] Anotherstudy in north India, showed out of 780 enterobacteriaceae, 64 isolates were phenotypically MBL producer. They also performed polymerase chain reaction (PCR)*and* 54 isolates were NDM producers which include 30 *Escherichia coli,* 12 *Citrobacter* spp. and 12 *Klebsiella* spp. with an overall occurrence of 6.9%.[18]

Carbapenemase producing bacteria can be detected by molecular or enzyme detection methods. Molecular methods are PCR, isoelectric focusing, spectrophotometry, colonic blot hybridization etc. Among them PCR is the most useful method with 100% sensitivity and specificity and time saving.[19]

Enzyme detection methods include modified hodge test or clover leaf test, double disk synergy test, disk test or disk potentiation test, EDTA-imipenem microdilution MIC test, E test MBL strip test etc. Double disk synergy tests (DDST) includes imipenem-EDTA double disk synergy test, ceftazidime-EDTA double disk synergy test, ceftazidime-1,10 phenanthroline double disk synergy test, ceftazidime-mercaptopropionic acid double disk synergy test, ceftazidime-mercaptoacetic acid double disk synergy test etc. Combined disc test or disk potentiation test includes imipenem and imipenem+EDTA combined disk test, ceftazidime and ceftazidime+EDTA combined disk test, carbapenem disk with and without a polyboronic acid test etc. Among the double disk synergy test imipenem-EDTA double disk synergy test is better and able to distinguish MBL producer from non-MBL producers . It is the most effective method for the detection of MBL producers with 77.9% sensitivity and 96% specificity.[20] The Combined disk test with imipenem & imipenem+EDTA is also very useful test and has sensitivity and specificity are 94.7%& 98 % respectively.

Now a day multidrug resistant gram negative bacteria are the greatest risk to public health. Gram negative bacteria develop resistant faster than gram positive bacteria.[21,22] There are few new antibiotics have developed and few are under process of developmenting.[23] But they may not be sufficient against gram negative bacteria to provide therapeutic cover after 10-20 years.[24,25,26] Thus MBL producing enterobacteriaceae are a challenge for wound infection management.

Methods

A total of 233 (79.79) enterobacteriaceae were isolated from 292 wound swabs in the microbiology laboratory of Rajshahi medical college during the period of January, 2014 to December, 2014. Standard method was employed for collection of swabs and cultured on neutrient agar and Mac Conkey's agar media. Enterobacteriaceae were

identified by their colonial morphology, gram staining, motility, oxidase , indole & urease production and citrate utilization tests. Sugars fermentation and H_2S production were done in triple sugar iron media. The sensitivity test was performed by modified Kirby Bauer disk diffusion method on Muller-Hinton agar media with meropenem (10g) and ceftriaxone (30g) disks. The resistant enterobacteriaceae were expressed by CLSI, 2012 recommendation. [27] The identified isolates which showed resistant to both meropenem and ceftriaxone were further tested for Metallo-beta-lactamase (MBL) production.[27,28] Metallo-beta-lactamase production was detected by double disk synergy test by putting imipenem (10g) and 10l of 0.5M-EDTA disks. The disks were placed 20 mm apart from each other on neutrient agar media and incubated aerobically at 37°c for 24 hours. The synergistic inhibition of zone indicate the production of MBL.[7,20,29]

Table 1. Resistant pattern of enterobacteriaceae against meropenem (10 µg) and ceftriaxone (30 µg) disks. (N=233)

Species	MEM resistant N(%)	CTR resistant N(%)	Both MEM & CTR N(%)
E.coli N=122	88(72.13)	113(92.62)	86(70.49)
Proteus spp. N=54	30(55.55)	44(81.48)	28(51.85)
Enterobacter spp. N=37	27(72.97)	33(89.19)	27(72.97)
Klebsiellae spp. N=14	8(57.14)	13(92.86)	8(57.14)
Providentia spp. N=6	3(50.00)	4(66.67)	3(50.00)
Total 233(100)	156(66.95)	207(88.84)	152(65.23)

C=meropenem; CTR =Ceftriaxone. Figures in parenthesis represent percentage.

Table 2. Detection of metallo-beta-lactamase production among isolates resistant to both MEM and CTR by double disk synergistic test.(N= 152).

Species resistant to both MEM & CTR	DDST N (%)
E.coli (N=86)	66(76.76)
Proteus spp. (N=28)	19(67.85)
Enterobacter spp. (N=27)	21(77.77)
Klebsiella spp. (N=8)	7(87.50)
Provindentia spp. (N=3)	1(33.33)
Total 152(100)	114(75.00)

Note: N=Number,DDST= Double disk synergy test, MEM = meropenem, CTR =Ceftriaxone

Result

Two hundred and thirty three enterobacteriaceae were isolated from 292 wound infections by culture and antibiogram was carried out to find meropenem and ceftriaxone resistant enterobacteriaceae. These resistant enterobacteriaceae were further studied for detection of metallo-beta-lactamase production which is responsible for resistant.

Table I shows resistance pattern of enterobacteriaceae against meropenem (MEM), ceftriaxone (CTR) alone and both meropenem & ceftriaxone. Out of 122 *E.coli*, 88(72.13%) & 113(92.62%) were resistant to MEM &CTR alone and 86(70.49%) were both MEM & CTR. Similarly among 54 *Proteus* spp., 37 *Enterobacter* spp, 14 *Klebsiella* spp. and 06 *Providentia* spp 30(55.55%), 44(81.48%) and28(51.85%) ; 27(72.97%) , 33(89.19%), and 27 (72.97%); 08(57.14%) , 13(92.86%) and 08 (57.14%); 03(50.00%) , 04(66.67%) and 3(50.00%) respectively. MEM resistant was less than CTR resistant when tested alone.

Table 2 shows the detection of metallo-beta-lactamase production from entrobacteriaceae isolates by double disc synergy test . Metallo-beta-lactamase was production was detected in 66(57.89%) species out of 86 resistant (MEM & CTR) isolates of *E.coli*. Similarly 19(16.67%) , 21(18.42%), 7(6.14%) and 1(0.88%) were detected from 28 *Proteus spp.*, 27 *Enterobacter spp.*,08 *Klebsiella* spp. and 03 *Providentia* spp. A total of 152(100%) different species entrobacteriaceae, 114(75.00%) species had produced MBL which was detected by DDST.

Discussion

Wound infection is a major problem in daily practice due to the emergence and spread of multidrug resistant bacteria specially enteterobacteriaceae which gaining more and more importance day by day. In this study the member of entrobacteriaceae which resistant to both meropenem and ceftriaxone were studied for metallo-beta-lactamase production by double disc synergy test and found *E.coli* was 66(57.89%), *Proteus* spp. 19(16.67%), *Enterobacter* spp. 21(18.42%), *Klebsiella* spp. 7(6.14%) and *Providential*spp. 1 (0.88%). Our study is dissimilar with the report by Haider *et al.* (2014) [30] in Uttar prodesh, India where they found *E.coli* was 36%, *Klebsiella* spp. 20%, *Proteus spp.* 8%, *Serratia* spp. 16% and *Citrobacter* spp. 20%. Dissimilarity was also reported by Naveenkumar *et al.*(2014)[29] in India where *E.coli* were 100% resistant to carbapenem. The dissimilarities may be due to the prevalence of MBL producing enteterobacteriaceae varies from country to country and also in different institution within the same country [31]. The dissimilarities may also be due to defective culture & sensitivity test, inadequate dose and duration of antibiotic used, sometimes the concentration of antibiotics may not be same as said by the pharmaceutical companies etc. Beside that other factors such as presence of geniticaly resistsnt strain, different geographical locations, environment, sanitation, habit of the patient and variation of antibiotics use in different hospitals.

We may conclude that multidrug resistant enterobacteriaceae was due to production of MBL as result of mutation of genes for misuse of antibiotics without during antibiogram. Enterobacteriaceae are the gut flora. So proper sewerage management may reduce wound infection caused by them. Antibiotic sensitivity test is mandatory before starting treatment. Every hospitals should have their own antibiotic policy, national guideline and some antibiotics should keep reserve for future use.

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