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Detection of metallo-β-lacatamase producing Bacteria in Clinical Isolates of patients using EDTA & Ceftazidime

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Abstract

The aim of present study is to check the antibacterial susceptibility of isolated bacterial species and to evaluate the Metallo β lactamase (MBL) production by Disk Potentiation Method and Double Disk Synergy Test using Ceftazidime and EDTA. A total of 101 clinical isolates of Gram Negative Bacilli were studied and among the isolates highest resistance was seen against Amoxycilin (92.22%) and least against Polymixin B (6.76%) followed by Imipenem (14.12%). A total of 35 (15.84%) isolates were found to be MBL producers. Among 35 positive isolates for MBL production, 29 (82.86%) were found to be positive by Disk Combination (Disk Potentiation) method and 21 (60%) were found to be positive by Disk Approximation (Double Disk Synergy) method. 100% concordance was shown among 15 (42.86%) isolates. Among 35 positive isolates, the top three bacterial species found to be MBL producers were E.coli, Klebsiella & Pseudomonas. A total of 3 (8.57%) positive isolates out of 35 were found to be sensitive for Ceftazidime.

Keyword: Antibacterial, ceftazidime , resistance.

Introduction:

Penicillin and other β-lactam antibiotics are drug of choice for treatment of bacterial infections for quite a long time, but the major problem with these are the gradual increase in antibiotic resistant strains of various bacteria. The main cause of this antibiotic resistance is the production of enzyme β-lactamases. Carbapenems are β-lactam antibiotics, presently considered as the most potent agents for treatment of multidrug resistant Gram-negative infections due to the stability of these agents against the majority of β-lactamases and their high rate of permeation through bacterial outer membranes (Gupta *et al*, 2006). β-lactam antibiotics include Penicillin & its derivatives, Cephalosporins, Monobactams, Carbapenems and any antibiotic that contains a β-lactam ring in its molecular structure.

Although carbapenem are most potent anti microbial agent for treatment of Multi Drug Resistant (MDR) Gram Negative Bacteria, some clinical Gram Negative Bacilli have been reported resistant to carbapenem due to production of enzyme carbapenemase or metallo-β-lactamase (MBL) (Agarwal *et al*, 2006; Varaiya *et al*, 2008). The emergence of MBL in gram negative bacilli possess a therapeutic challenge as MBL production causes high degree of hydrolytic cleavage of higher Cephalosporins and treatment alternatives are unavailable or are expensive/toxic with poor outcome (Irfan *et al,* 2008).

Pseudomonas aeruginosa producing MBL was first reported from Japan in 1991 and since then has been described from various parts of the world, including Asia, Europe, Australia, South America, and North America. These MBLs belong to Ambler class B and have the ability to hydrolyze a wide variety of β-lactam agents, such as penicillins, cephalosporins, and carbapenems. These enzymes are inhibited by metal chelators, such as EDTA and thiol-based compounds (Pitout *et al*, 2005).

The present study is carried out in order to calculate prevalence of MBL in clinical isolates of patients.

Materials and Methods:

A total of 101 isolates of gram negative bacteria samples were collected from Jan 5, 2009 to May 15, 2009 from the patients admitted to various specialties of associated tertiary Hospital of Uttarakhand Forest Hospital Trust Medical College, Haldwani.

Identification of Organisms:
The bacterial pathogens were identified by conventional biochemical methods according to standard microbiological techniques (Collee *et al*, 1996) which include:

1. Gram Staining
2. Biochemical Tests

If no bacterial growth in sample was observed on Mac Conkey agar and Blood agar incubated at 37^0C for 24 hours, then sub-culture was done. Sample was considered sterile if there was no growth on sub-culture.

Antibacterial Susceptibility Testing:
Antibacterial sensitivity was performed on Mueller Hinton agar (Hi Media, India) by the standard disc diffusion method recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2002). The following 22 antibiotics (Hi media, India) were tested for antibacterial susceptibility:
Ampicillin, Amoxycilin, Amoxycilin/Clavulanic Acid, Ampicillin/Sulbactum, Gentamicin, Amikacin, Cephotaxime, Ceftriaxone, Cefixime, Cefazonil, Gatifloxacin, Levofloxacin Ciproflaxin, Ofloaxin, Co-Trimoxazole, Chloramphenicol, Erythomycin, Piperacilin/Tazobactum, Polymixin B, Imipenem, Ceftazidime, Tetracyclin, Ceftazidime/Clavulanic Acid and Netilin.
The diameter of the zone of inhibition of growth was recorded and interpreted as susceptible or resistant by the criteria of NCCLS (NCCLS, 2002). Organisms with intermediate level of resistance were included in the percentage of resistance organisms for final analysis.

MBL Detection:
In this study following two methods were involved for detecting MBL production in Gram Negative isolates (Behera *et* al, 2008):

1. Disk Potentiation Test (Disk Combination Method; DCM)
2. Double Disk Synergy Test (Disc Approximation Method; DPM)

In Disk Potentiation method, a lawn culture of test organism was prepared. Then two 30 µg Ceftazidime disks were kept at some distance apart, with one disk containing about 5 µl of 0.5 M EDTA solution. After the incubation of overnight at 37^0C, production of MBL by the test organism is inferred by the increase in size of zone of inhibition by >7mm around the disk containing EDTA.

In Double Disk Synergy Test, the test organisms were inoculated onto Muller Hinton agar (Hi Media, India) plate. Then two of Ceftazidime disks (30 µg) were placed at distance of 20mm and 15 mm (center to center) from EDTA containing disk (4µl/disk). Then plates were kept for overnight incubation at about 37^0C. The presence of even a small synergistic inhibition zone is interpreted as positive for MBL production.

Results and Discussion:
Out of total of 101 bacterial isolates, *E.coli* was the pre-dominant species (56.44%) followed by *Klebsiella* spp. (14.85%) and *Pseudomonas* (12.87%). Present study demonstrated high level resistance to commonly used antibiotics. Highest resistance was seen against amoxycilin (92.22%) and least by polymixin B (6.76%) followed by imipenem (14.12%). Another study from Delhi by Mohanty *et al,* 2004 also observed higher level of resistance among isolates. This higher level of resistance may be due to rampant and injudicious use of antibiotics.

Table:1 Total Number of Organism Of Each Species Detected As MBL Producers

Species (No.)	Detection Method		Common	Total Detections
	DCM	**DPM**		
E.coli (57)	16 (76.19%)	12 (57.14%)	7 (33.33%)	**21 (36.84%)**
Pseudomonas (13)	4 (80%)	3 (60%)	2 (40%)	**5 (38.46%)**
Acinetobacter (6)	2 (100%)	1 (50%)	1 (50%)	**2 (33.33%)**
P.mirabilis (3)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Klebsiella (15)	6 (100%)	5 (83.33%)	5 (83.33%)	**6 (40%)**
Citrobacter (2)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
M.morganii (1)	1 (100%)	0 (0%)	0 (0%)	**1 (100%)**
Enterobacter (2)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Salmonella (2)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Total (101)	**29 (82.86%)**	**21 (60%)**	**15 (42.86%)**	**35 (34.65%)**

The detection of MBL producing isolates ranges from 7% (Navneeth *et al,* 2002) to 100% (Walsh *et al*, 2002) in other studies. Out of 101 isolates, 35 (34.65%) were found to be positive for MBL production. Of these disk combination detected 29 (28.71%) and disk approximation detected 21 (20.79%) MBL producing strains. Among the 35 isolates, 15 (14.85%) of them shown 100% concordance by both these methods. 14 (40%) isolates found to be positive by disk combination but negative by disk approximation. Similarly 6 (17.14%) isolates found to be positive by disk approximation but negative by disk combination. Out of 35 isolates, *E.coli* (*60%*), *Pseudomonas* (14.29%), *Acinetobacter* (5.71%), *Klebsiella* (17.14%) and *Morgnella morganii* (2.86%) were found to be MBL producers. Among 29 isolates detected as positive for MBL production by disc combination method, 26 (89.66%) were resistant to ceftazidime.

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