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PHENOTYPIC DETECTION OF METALLO-β-LACTAMASE PRODUCING ENTEROBACTERIACEAE

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Abstract-

Aim: Phenotypic Detection of Metallo-β-lactamase-producing Enterobacteriaceae from Patients of a Tertiary Care Hospital, Ahmedabad.

Material and Method: The study was conducted over period of one year, from July 2011 to June 2012.

A total of 1072 Enterobacteriaceae isolates from various clinical samples of indoor patients were included in the study. All isolates were non-duplicate. Antimicrobial susceptibility of all the isolates was performed by the disc diffusion method. Metallo beta lactamase (MBL) production was detected in imipenem-resistant isolates by phenotypic tests. The Imipenem (IMP)-EDTA combined disc diffusion test was used.

Result and Discussion: MBL producing Enterobacteriaceae isolates were 2.35%. Most common MBL producing organism was *Klebsiella pneumoniae*, from swab and urine of patients collected from ICU (debilitated patients). In present study, the imipenem-resistant isolates also show resistance to other groups of antibiotics, which is a uniquely seen with MBLs producers that show a broad-spectrum resistance profile. The majority of these MBL isolates were from patients of the intensive care unit (ICU) and post-operative wards (surgical ward); areas where the majority of critically ill patients are concentrated. The majority of the organisms were from swab and urine. *Klebsiella pneumoniae* among all Enterobacteriaceae were the predominant MBL producers in our study.

Conclusion: There is a need for active surveillance to detect MBL producers. There should be judicious use of carbapenems to prevent their spread and use of effective antibiotics as per the antibiotic-sensitivity report.

Keywords- Enterobacteriaceae, imipenem, metallo-β-lactamases, carbapenemases, disc diffusion test, multi drug resistance

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Introduction

The increase in the rates of antibiotic resistance is a major cause for concern in isolates of the Enterobacteriaceae family. B-lactams have been commonly used for treatment of serious infections. Higher drugs like carbapenems, are advocated for the treatment of infections caused by extended-spectrum-β-lactamase (ESBL)-producing Enterobacteriaceae, common ESBL producers are *Escherichia coli* and *Klebsiella pneumonia* against which carbapenems is active [1].

Acquired metallo-β-lactamases (MBL) have recently emerged as one of the most worrisome resistance mechanisms owing to their capacity to hydrolyze all β-lactams, including carbapenems. Such strains are also not susceptible to therapeutic serine β-lacatamase inhibitors (such as clavulanate and sulfones). Moreover, MBL genes are carried on highly mobile elements, allowing easy dissemination. MBLs have been categorized into two major groups: Imipenemases (IMP) and Verona imipenemases (VIM).Others are German imipenemases (GIM) and Seoul imipenemases (SIM). They do not hydrolyze aztreonam. Most commonly seen in *P. aeruginosa, A. baumannii* and Enterobacteriaceae.

MBLs can be either chromosomally or plasmid mediated [3-12]. Although MBL-producing organisms have been detected in many parts of the world the exact prevalence rates in these countries remain unclear. Invasive infections with MBL-producing isolates are also associated with a higher morbidity and mortality [2]. The occurrence of an MBL-positive isolate in a hospital environment poses not only a therapeutic problem but is also a serious concern for infection control management. In recent years, MBL genes have spread from *P. aeruginosa* to members of the Enterobacteriaceae [3,4].

Five different types of MBLs whose prevalence are increasing rapidly are IMP, VIM, SPM, GIM and SIM. Among these, IMP and VIM

are the most predominant. With the global increase in the occurrence of MBLs, early detection is crucial, the benefits of which include timely implementation of strict infection control and treatment with alternative antimicrobials [4]. Molecular techniques are available to detect MBL production, for which IMP-EDTA combined disc test is sensitive and specific. According to Yong, et al., the IMP 10µg-EDTA 750µg combined disc test for detection of metallo-betalactames in MBL producing Enterobacteriaceae, with 80% sensitivity and 100% specificity is used [5].

Phenotypic assay for detection of MBL and Carbapenemases producing *Klebsiella pneumonia* (KPC) in *Klebsiella pnemoniae have been used*. These tests distinguished accurately between several different mechanisms mediated reduced susceptibility for carbapenems in Enterobacteriaceae. EDTA has excellent sensitivity for detection of MBL producing *Klebsiella pnemoniae* [3]. Among these carbapenemases especially transferrable metallo beta lactamases are most important because of their ability to hydrolyze virtually all drugs in that class including carbapenems.

It was found a very high prevalence of multidrug-resistant (MDR) and ESBL-positive gram negative bacteria in intensive care units (ICUs) and other wards. Carbapenems and cephalosporin/inhibitor combinations are being used as the "last resort" in infections occurring in critically ill patients, since last few years. Theirs a global increase in the prevalence of MBL-producing non-fermenting bacilli and Enterobacteriaceae [2,5-8]. So we have undertaken this study to find the prevalence of MBL-producing Enterobacteriaceae in our hospital.

Materials and Methods

The study was conducted from July 2011 to July 2012.

A total of 1072 Enterobacteriaceae isolates from various clinical samples of indoor patients were included in the study. All isolates were non-duplicate. Antimicrobial susceptibility of all the isolates was performed by the disc diffusion method [17]. Enterobacteriaceae were tested for following antibiotic panel, by the disk diffusion method: Ampicillin (20ug), Cotrimoxazole(25ug), Gentamicin (10µg), Cefotaxime (30µg), Ceftriaxone (30µg), Ceftazidime/ Clavulanic acid(30µg/10µg), Cefaclor(30ug), Cefipime(30ug), Tetracycline(30ug), Amikacin(30ug), levofloxacin(5ug) and Imipenem (10µg), piperacillin/tazobactam (100µg/10µg).

MBL production was detected in imipenem-resistant isolates by phenotypic tests. The IMP-EDTA combined disc diffusion test was used.

The IMP-EDTA combined disk test was performed as described by Young, et al. Test organisms were inoculated on to plates of Mueller Hinton agar as recommended by the CLSI [10]. Two 10-µg imipenem disks were placed on the plate and appropriate amounts off 10 µL of EDTA solution was added to one of them to obtain the desired concentration (750µg). The inhibition zones of the imipenem and IMP-EDTA disks were compared after 16-18 h of incubation in air at 35°C. In the combined disc test, if the increase in inhibition zone with the IMP and EDTA disc was ≥5 mm than the imipenem disc alone, it was considered as MBL positive. The isolates that are IMP resistant and not showing MBL production were tested for Modified Hodge test [12] to detect other mechanisms of carbapenem resistance.

Results

Out of 1072 Enterobacteriaceae isolates, 250 isolates were MDR, 68 showed imipenem resistance. A total of 40 isolates showed MBL production by the IMP-EDTA combined disc test.

[Table-1] shows the organism wise distribution of MBL production.

Table 1- MBL producing organisms.

Organism	Number and Percentage of MBL Producers
Klebsiella pneumonia	21(52.5%)
E. coli	9(22.5%)
Proteus spp.	6(15%)
Providentia spp.	4(10%)

[Fig-1] shows the 40 MBL producers, 21(52.5%) *Klebsiella pneumonia, 9(22.5%) E. coli, 4(10%) Providentia spp.* and 6(15%) *Proteus* species.

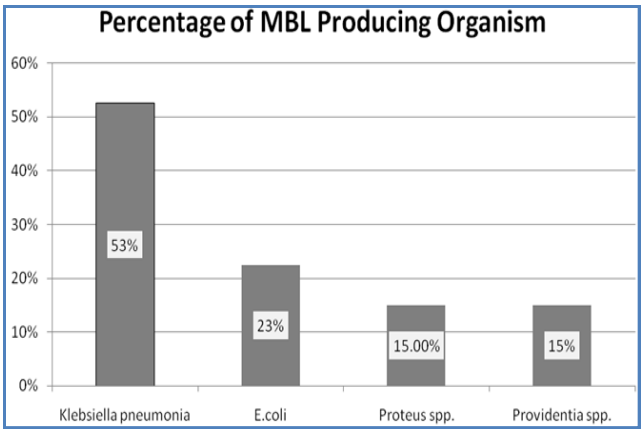

Fig. 1- Percentage of MBL producing organisms

[Table-2] mentions the distribution of MBL producers in various wards of the hospital during the study

Table 2- Ward wise distribution of MBL producers

Clinical Ward	Number and Percentage of MBL Production
Surgical ward	14(35%)
Orthopaedic ward	8(20%)
Paediatric ward	7(17.5%)
Medical ward	6(15%)
Gynaec ward	5(12.5%)

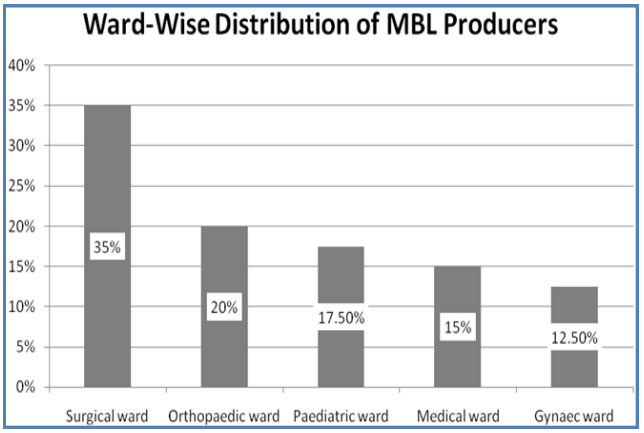

Fig. 2- Ward wise distribution of MBL producers

[Fig-2] shows the Location-wise distribution shows that 14(35%) isolates were from surgical ward, 6(15%)were from medical ward, 7 (17.5%) were from pediatric ward, 5(12.5%) were from gynaec ward, 8(20%) from orthopaedic ward. Out of these 23(57.5%) isolates were from the ICU.

[Table-3] shows the distribution of the MBL producers in various specimens received during study

Table 3- Sample wise distribution of MBL producers

Sample	Number and Percentage of MBL Production
Swab sample	17(42.5%)
Urine sample	14(35%)
Pus sample	9(22.5%)
Blood samples	5(12.5%)

[Fig-3] shows that out of 40 isolates showing MBL production, 9 (22.5%) were from the pus samples, 5(12.5%)from blood samples,17(42.5%)from swab samples, 14(35%) from urine samples.

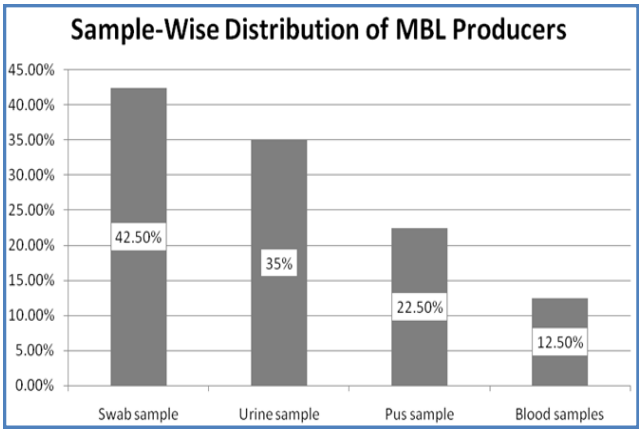

Fig. 3- Sample wise distribution of MBL producers

Out of 40 imipenem-resistant Enterobacteriaceae, 27 (67.5%) isolates were resistant to all the drugs tested, while 13(32.5%) were sensitive to levofloxacin. 4(10%) were sensitive to Amikacin. All isolates were resistant to Ampicillin, Gentamicin Piperacillin, Piperacillin/Tazobactam, cotrimoxazole, tetracycline, Cefotaxime, Ceftriaxone, ceftazidime and cefepime.

MBL producing Enterobacteriaceae isolates were 2.35%. Most common MBL producing organism was *Klebsiella pneumonia*, isolated from swab and urine of patients admitted in ICU (debilitated patients).

Discussion

In our study, MBL producing Enterobacteriaceae was 2.35%. They were found to be Multi drug resistance. The genes encoding MBLs commonly IMP gene and VIM gene are often procured by class 1 (and sometimes class 3) integrons. Integrons are embedded in transposons, resulting in a highly transmissible genetic apparatus that can be transferred between bacteria [2]. MBL producing Enterobacteriaceae confer resistance to other antibiotics such as fluoroquinolones, aminoglycosides and co-trimoxazole.

MBL producing organisms were isolated mainly from critically ill and debilited patients admitted in ICU and post operative (Surgical) ward. Use of indwelling medical devices is common in these areas, which play an important role in the spread of infective agents and also the injudicious use of antibiotics which confers resistance to higher drugs. The majority of the organisms were isolated from swab and urine. *Klebsiella pneumoniae* among all Enterobacteriaceae were the predominant MBL producers in our study.

These MBL producers are susceptible only to colistin, aztreonam, tigecycline and polymyxin except *Proteus* spp. which are inherently resistant to polymyxin.

The proportions of MBL-producing Enterobacteriaceae isolates from the National Cheng Kung University which was (2.9%) in *E. cloacae* isolates confirmed by *bla*IMP-8 colony hybridization, PCR and sequence analysis which is comparable to our study i.e. 2.35%. All MBL-producing isolates were found to be resistant to ceftazidime, cefotaxime, cefoxitin, cefepime, chloramphenicol, trimethoprim-sulfamethoxazol and aminoglycoside and this resistance phenotypes was transferred to their transconjugants, suggesting that the transferred plasmids also contained genetic determinants responsible for resistance to the non-beta-lactam antimicrobial agents [14]. The study of outbreak from Italy was caused by VIM-1 MBL gene and also an SHV-type ESBL gene [15].

The treatment option can be a combination with a carbapenem or an active aminoglycoside. The therapeutic options for treating infections due to MBL-producing isolates are limited. Unfortunately, emergence of colistin resistance in Enterobacteriaceae has been described in the literature in sporadic cases [16,17], as well as in multiclonal clusters in ICU patients [18], as a result of selective pressure from colistin use. Hence overuse of colistin should be checked. The in vitro activity of tigecycline against MBL-producing organisms was documented in the current study [19]. Awareness and early detection of these emerging pathogens, wiser antibiotic policies and stricter implementation is required which could limit their spread in the hospital.

Conclusion

Emergence of MBL producing enterobacteriaceae is alarming and reflects the excessive use of carbapenems. Therefore, early detection and prompt instillation of infection control measures is important to prevent further spread of MBLs. It is also important to follow antibiotic restriction policies to avoid excessive use of carbapenems and other broad-spectrum antibiotics. There is a need for active surveillance to detect MBL producers. There should be judicious use of carbapenems to prevent the spread of resistance and use of effective antibiotics as per the antibiotic-sensitivity report. Colonization with an MBL-producing Enterobacteriaceae can cause severe, often fatal infection in severely ill patients. Both infection control practices and antibiotic policies should be intensified to contain the spread of these problematic bacteria.

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