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Research Paper

PHENOTYPIC DETECTION AND PREVALENCE OF METALLO β-LACTAMASES (MBLs) IN CARBAPENEM RESISTANT ISOLATES OF *ACINETOBACTER* SPECIES AT A TERTIARY CARE HOSPITAL IN NORTH INDIA

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Metallo-β-lactamase (MBL) producing *Acinetobacter* species has become a growing therapeutic concern worldwide. Thus, the present study was conducted to determine the prevalence of MBLs in imipenem-nonsusceptible isolates of *Acinetobacter* species from a teaching tertiary care hospital. During a period of one year (May 2010 to April 2011), 100 isolates of *Acinetobacter* species were collected from different clinical specimens received in Microbiology laboratory. All isolates were tested for antimicrobial susceptibility by Kirby-Bauer disc diffusion method. Carbapenem nonsusceptible isolates were further processed for detection of MBL production by combined disc test according to Yong et al. Of the 100 *Acinetobacter* isolates, 82 were identified as *A. baumannii* and 18 as *A. lwoffii*. Imipenem nonsusceptible isolates were 70%, out of which 39 (55.7%) isolates showed MBL production. Of 39 MBL positive isolates, thirty four (87.1%) were multi drug resistant. Statistically significant difference was found in the antimicrobial resistance of MBL producing and MBL non producing isolates for ciprofloxacin, amikacin, gentamicin, netilmicin, piperacillin tazobactam and β-lactam antibiotics (p value < 0.05).

Keywords: Acinetobacter baumannii, Carbapenems, Metallo-β-lactamases, Multidrug resistance

INTRODUCTION

Acinetobacter baumannii is the most important species of genus *Acinetobacter* which has emerged as one of the significant nosocomial pathogen worldwide. Species other than *A. baumannii* such as *A. lwoffii*, *A. johnsonii*, *A. junii* and *A. haemolyticus* are involved less frequently in nosocomial infections, and are generally more susceptible to antibiotics. *A. baumannii* causes a wide range of clinical infections such as pneumonia, blood stream infections, urinary tract infections, wound infections and meningitis especially in patients admitted in intensive care units. The treatment of these infections is hampered by the rapid rise in prevalence of *A. baumannii* strains that are resistant to almost

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all available antibiotics, including β-lactams, fluoroquinolones, tetracyclines, and aminoglycosides (Bergogne and Towner, 1996). Carbapenem group of antibiotics play a vital role in the management of infections caused by these multi drug resistant (MDR) strains, because of their broad spectrum activity and stability to hydrolysis by most of the b-lactamases, including extended spectrum β-lactamases. However, now a days carbapenem resistance in *Acinetobacter* is observed increasingly worldwide. Metallo-β-lactamases (MBLs) confer a high level of carbapenem resistance in *A. baumannii* isolates, as well as resistance to all β-lactams except aztreonam, because of their strong hydrolytic activity against these antibiotics. MBL producing isolates also have the propensity to show concomitant resistance to multiple antimicrobial agents thus further limiting therapeutic options (Poirel and Nordmann, 2006). This necessitates rapid screening and detection of MBLs in *Acinetobacter*, so as to modify therapy and initiate effective infection control measures to prevent further dissemination of this catastrophic agent. Therefore, an attempt have been made in the present study to identify phenotypically metallo-β-lactamases in carbapenem resistant isolates of *Acinetobacter* species.

MATERIALS AND METHODS

Study Design

The present prospective study was conducted at the Microbiology Department of a teaching tertiary care hospital over a period of one year (May 2010 to April 2011). A total of hundred non-duplicate, non-consecutive isolates of *Acinetobacter* species were included in the study. These strains were isolated from various clinical samples collected aseptically from patients of all age groups and sex, admitted in intensive care units (ICUs), wards and outdoor Departments of our hospital. The samples included were sputum, endotracheal aspirates, bronchoalveolar lavage (BAL), blood, pus, urine, cerebrospinal fluid (CSF), wound swabs, high vaginal swabs (HVS), throat swabs and other body fluids.

Identification and Antimicrobial Susceptibility Testing

All the samples were routinely cultured on blood agar and MacConkey agar. After overnight incubation at 37^0C, the suspected colonies were further processed for identification of *Acinetobacter* species by Gram staining, oxidase test, hanging drop and by other standard biochemical tests (Collee *et al.*, 1996). Antimicrobial susceptibility testing was done on Mueller Hinton agar by disc diffusion method and results were interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines. The following antimicrobial discs (Hi-media, Mumbai, India) with their concentration given in parenthesis were used. ceftazidime (30 μg), cefepime (30 μg), ceftriaxone (30 μg), cefotaxime (30 μg), amoxycillin/clavulanic acid (20 μg/10 μg), imipenem (10 μg), piperacillin/tazobactam (100 μg/10 μg), ticarcillin/clavulanic acid (75 μg/10 μg), gentamicin (10 μg), amikacin (30 μg), netilmicin (30 μg), ciprofloxacin (5 μg), doxycycline (30 μg), cotrimoxazole (25 μg), polymyxin B (300 units) and colistin (10 μg). American Type Culture Collection (ATCC) strain viz. *Escherichia coli* 25922 was employed as a control strain.

Detection of MBL Production

Isolates showing reduced susceptibility to imipenem (zone diameter <16mm) were further processed for MBL production by combined disc test according to Yong *et al*, 2002 with slight

modification. A 0.5M EDTA solution was prepared by dissolving 18.61 gm of disodium EDTA.2H_2O in 100ml of distilled water and adjusting it to pH 8 by using NaOH. Two 10 μg imipenem discs and two 30 μg ceftazidime discs (15 mm from edge to edge) were placed on the surface of agar plate inoculated with test organism and EDTA solution (10 μl) was added to one of them to obtain a desired concentration of 750 μg. The inhibition zones of imipenem, ceftazidime and imipenem EDTA and ceftazidime EDTA discs were compared after 16-18 h of incubation at 37^{0}C. A positive test was indicated by zone enhancement with EDTA impregnated imipenem and ceftazidime discs. The zone size enhancement of ≥5 mm for ceftazidime EDTA disc as compared to ceftazidime alone and a zone size enhancement of ≥7 mm for imipenem EDTA disc as compared to imipenem alone was taken as positive criteria for MBL production.

Statistical Analysis

For comparison of two or more set of variables, p value was calculated by using SPSS version 19. If the p-value was <0.05, it was considered significant.

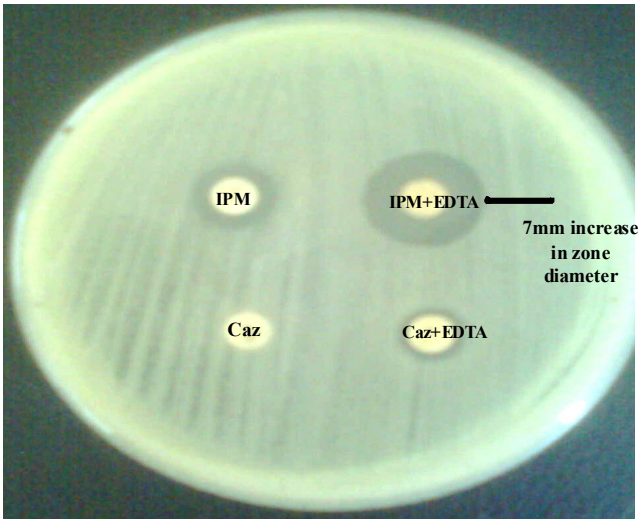
RESULTS

Of the 100 *Acinetobacter* isolates (*A. baumannii* = 82, *A. lwoffii* = 18), 70% were imipenem resistant, out of which 39 (55.7%) isolates showed MBL production by combined disc test (Table 1) (Figure 1). Of 39 MBL producing *Acinetobacter* isolates, 15 showed zone enhancement with both

Table1: Distribution of MBL Producing *Acinetobacter* Isolates Among Imipenem Resistant (Screen Positive) Isolates

Acinetobacter Species	Imipenem Resistant Isolates n(% Resistance)	MBL Producers n(%)
A. baumannii (n=82)	66(80.4)	38(57.5)
A. lwoffii (n=18)	4(22.2)	1(25)
Total (n=100)	70(70)	39(55.7)

Figure 1: MBL production in *Acinetobacter* Species
IPM=imipenem, IPM+EDTA=imipenem+EDTA, Caz=ceftazidime, Caz+EDTA=ceftazidime+EDTA



imipenem and ceftazidime EDTA combinations, 13 isolates showed zone enhancement with ceftazidime EDTA only and 11 isolates showed zone enhancement with imipenem EDTA only. Maximum number of MBL production was detected in isolates obtained from respiratory samples 17/39 (43.5%) and blood 15/39 (38.4%). Majority of the MBL producing isolates were from ICU patients 19/39 (48.7%), followed by patients admitted in different wards 14/39 (35.8%) and outdoor patients 6/39 (15.3%). Out of 39, twenty eight (71.7%) were from adult patients and 11 (28.2%) were from children. Of 39 MBL positive isolates, thirty four (87.1%) were MDR. Statistically significant difference was found in the antimicrobial resistance of MBL producing and non producing isolates for ciprofloxacin, aminoglycosides, piperacillin tazobactam, ticarcillin clavulanic acid, amoxyclav and β-lactams (p value <0.05) (Table 2). None of the isolate was resistant to polymyxin B and colistin.

DISCUSSION

Carbapenem resistance in *Acinetobacter* species is a growing concern now a days since it limits the therapeutic options. Only few drugs such as colistin and tigecycline are suggested as possible effective treatment choices against carbapenem resistant isolates. The present study showed high level of imipenem resistance (70%) among *Acinetobacter* species. Numerous Indian studies from different regions have documented low (9%) to very high (90%) carbapenem resistance in *Acinetobacter* isolates (Taneja *et al.*, 2003; Gaur *et al.*, 2008; Karthika *et al.*, 2009). The higher resistance to carbapenems in our study may be attributed to increased use of carbapenems in our hospital to treat MDR *A. baumannii* infections

TABLE 2: Comparison of the Antimicrobial Resistance of MBL Producing and MBL Non Producing *Acinetobacter* Isolates

Antimicrobial Drugs	MBL Producers (n=39)		MBL Non Producers (n=31)		*P* Value
	n	Resistance (%)	n	Resistance (%)	
Ciprofloxacin	30	76.9	17	54.8	<0.05
Amikacin	30	76.9	16	51.6	<0.05
Gentamicin	34	87.1	21	67.7	<0.05
Netilmicin	27	69.2	14	45.1	<0.05
Ticarcillin+clavulanic acid	38	97.4	21	67.7	<0.05
Piperacillin+tazobactam	25	64.1	12	38.7	<0.05
Amoxycillin+clavulanic acid	38	97.4	22	70.9	<0.05
Cotrimoxazole	35	89.7	20	64.5	<0.05
Doxycycline	33	84.6	13	41.9	<0.05
Ceftazidime	37	94.8	26	83.8	>0.05
Ceftriaxone	39	100	26	83.8	<0.05
Cefotaxime	37	94.8	24	77.4	<0.05
Cefepime	39	100	24	77.4	<0.05

especially in ICUs leading to selection of resistant isolates in the hospital. *A. lwoffii* is usually considered to be more susceptible to antibiotics and less pathogenic than *A. baumannii*. The present study showed significant difference (*p* value <0.01) in terms of susceptibility to imipenem between *A. baumannii* (19.5%) and *A. lwoffii* (77.7%). Study from USA (Jones *et al.*, 1999) showed that 96.7% isolates of *A. lwoffii* were imipenem susceptible. Noyal *et al*, 2009 from India also reported a difference in resistance to meropenem between *A. baumannii* (59%) and *A. lwoffii* (31.8%). In our study, metallo-β-lactamases were detected in 57.5% and 25% of the imipenem resistant isolates of *A. baumannii* and *A. lwoffii* respectively by combined disc test. In CLSI guidelines, no phenotypic method has been given for screening and confirmatory detection of MBLs in *Acinetobacter* species. Therefore, different phenotypic methods have been used by different authors from Indian subcontinent to detect MBLs in *Acinetobacter* species. We used combined disc test as it is sensitive, specific and easy to carry out in small laboratories. Karthika *et al.* (2009) have reported MBL production in 70.9% of imipenem resistant isolates of *Acinetobacter baumannii*. Anwar *et al.* (2010) have reported MBL production in 65.5% of the imipenem resistant *Acinetobacter* isolates which was higher than the present study. The non detection of MBLs in 44.2% of the carbapenem resistant isolates in our study could be due to the fact that apart from MBL production, carbapenem resistance in *Acinetobacter* spp. can occur due to some other resistance mechanisms. *Acinetobacter* spp. inherently possess oxacillinases represented by OXA-51/69 and AmpC type cephalosporinases, overexpression of which leads to carbapenem resistance and these enzymes are difficult to be detected by simple laboratory methods. Along with these intrinsically occurring enzymes, *Acinetobacter* spp. also possess carbapenem hydrolyzing class D β-lactamases (CHDLs) represented by OXA-23, 27, 49, 24, 25, 26, 40 and 58 which are acquired β-lactamases and responsible for carbapenem resistance. Reduced expression of outer membrane proteins, modification of the penicillin binding proteins and efflux mechanisms also contribute to carbapenem resistance in *Acinetobacter* spp. As these resistance mechanisms were not studied, so, these might have contributed to carbapenem resistance in non MBL producing *Acinetobacter* isolates in our study.

By using ceftazidime EDTA along with imipenem EDTA, we were able to detect additional isolates showing MBL activity as thirteen isolates showed zone enhancement with ceftazidime EDTA only. The increased sensitivity of EDTA ceftazidime combination have also been reported by Noyal *et al*, 2009 and Hemalatha *et al*, 2005. These results indicate the ability of ceftazidime to produce a marked inhibitory effect with EDTA. In the current study, 87.1% MBL positive isolates were MDR and on comparing the antimicrobial resistance pattern of MBL producing and non producing isolates of *Acinetobacter* spp., we observed that MBL producing isolates also showed concomitant resistance to aminoglycosides and fluoroquinolones. The p value was found to be <0.05 which indicated a significant difference. Peymani *et al.*, 2011 also found that all MBL producing isolates were MDR and exhibited high degree of resistance to β-lactams, aminoglycosides and fluoroquinolones. Similar results have also been noted by Anuradha *et al.*, 2010. Since, MBL genes are located on plasmids and these plasmids also carry genes

causing resistance to other antibiotics such as aminoglycosides and fluoroquinolones, therefore, coresistance to other antibiotics are common in MBL producing isolates (Walsh *et al.*, 2005).

CONCLUSION

Hence, by acquiring metallo-β-lactamases, *Acinetobacter* strains showed high level of resistance to β-lactam antibiotics. These isolates also showed significant coresistance to other class of antibiotics thus, leaving behind only few therapeutic options. Failure to detect these enzymes has contributed to their uncontrolled spread and therapeutic failures. So, these β-lactamases should be detected routinely in clinical laboratories by using appropriate methods and reported to clinicians at time so that inappropriate use of antibiotics can be stopped in time.

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