Int J Pharm Bio Sci 2013 Apr; 4(2): (B) 580 - 585

Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

INCIDENCE OF METALLO-β-LACTAMASE (MBL) PRODUCING NONFERMENTERS ISOLATED FROM RESPIRATORY SAMPLES IN ICU PATIENTS

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ABSTRACT

Nonfermenting gram negative bacilli (NFGNB) cause serious infections in immuncompromised and hospitalized patients. They cause fatal lower respiratory tract infections in patients admitted to ICU's. The appearance of metallo ß-lactamases (MBL) genes and their spread among bacterial pathogens is a matter of concern. The present study was undertaken to know the prevalence of MBL producing NFGNB.NFGNB isolated from respiratory samples of patients admitted in various ICU's were identified. The antimicrobial susceptibility testing was done as per CLSI guidelines.. Imipenem resistant isolates were selected for the detection of MBL production by Imipenem-EDTA combined disc test. A total of 445 NFGNB were isolated, *Acinetobacter baumannii* was the most common isolate followed by *Pseudomonas aeruginosa*. Piperacillin/tazobactam and imipenem were the most effective antibiotics. 40 % of *A. baumannni* and 20% of *P.aeruginosa* isolates showed resistance to imipenem. MBL production was detected in 80.1% isolates. To conclude, carbapenem resistance and MBL production in NFGNB is not uncommon.

KEYWORDS: **Carbapenem resistance, metallo ß-lactamases, NFGNB, ICUs**

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INTRODUCTION

Nonfermenting gram-negative bacilli (NFGNB) are a taxonomically diverse group of aerobic, nonsporing bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively[1]. They occur as saprophytes in the environment and some are also found as commensals[2, 3]. NFGNB are known to account for about 15% of all bacterial isolates from a clinical microbiology laboratory [4]. In recent years, due to the liberal and empirical use of antibiotics, NFGNB have emerged as important healthcare-associated pathogens. They have been incriminated in infections, such as, septicemia, meningitis, pneumonia, urinary tract infections (UTI), and surgical site infections (SSI) [3]. NFGNB are innately resistant to many antibiotics and are known to produce extended spectrum ß-lactamases and metallo ß-lactamases [3, 4]. NFGNB causes serious infections in immunocompromised and hospitalized patients especially those admitted to intensive care units (ICU). These organisms further worsen the situation by virtue of their multidrug resistance and thus limit therapeutic options [5]. Carbapenems first introduced in 1980 are now frequently used as the last choice in treating serious infections caused by multidrug resistant gram negative bacilli. These antibiotics are stable to ß-lactamases including the extended spectrum ß- lactamases (ESBLs) and Amp C produced by gram negative bacilli. Unfortunately resistance to these antibiotics started emerging from 1990 and has been reported in nonfermenting gram negative bacilli (NFGNB) worldwide over the years with varying frequencies [6-8]. *Pseudomonas aeruginosa* and *Acinetobacter* spp. in particular are most often associated with carbapenem resistance. Unfortunately, there is paucity of data on the prevalence of carbapenem resistance in the Indian literature[9]. Reportedly, several outbreaks due to carbapenem resistant NFGNB have resulted with considerable morbidity and mortality[10, 11]. Resistance to these antibiotics is due to decreased outer membrane permeability, increased efflux systems, alteration of penicillin binding proteins and carbapenem hydrolyzing enzymes – carbapenemases. These carbapenemases may be class B-metallo □-lactamases (IMP,VIM) or class D-oxacillinases (OXA-23 to OXA-27) or class A- clavulanic acid inhibitory enzymes (SME, NMC, IMI,KPC) [12]. They may be chromosomally or plasmid mediated and therefore pose a threat of spread of resistance by gene transfer among gram negative bacteria. The appearance of MBL genes and their spread among bacterial pathogens is a matter of concern. The present study was undertaken to find the prevalence of MBL producing NFGNB isolates from respiratory samples

MATERIALS AND METHODS

This was a prospective study for a period of six month on patients admitted in intensive care units with clinical evidence of lower respiratory tract infections. All the respiratory specimens were processed by standard methods[1]. NFGNB isolated from respiratory samples were identified by the colony characterstics, motility, oxidase test. Further identification was done by various tests like OF-glucose/mannitol, arginine dihydrolase, lysine decarboxylase, polymyxin B sensitivity, growth in 6.5% NaCl, growth at 44°C and 42°C, citrate utilization and indole production[1]. The antimicrobial susceptibility testing was done by Kirby Bauer disc diffusion method using gentamicin (10 µg), amikacin (30 µg), cotrimoxazole (1.25/23.75µg), ciprofloxacin (5µg), ceftazidime (30 µg), cefepime (30µg), piperacillin (100µg), piperacillin/tazobactam (100/10µg), and imipenem (10 µg) as per CLSI Guidelines[13]. Imipenem resistant isolates were selected for the detection of MBL production by Imipenem-EDTA combined disc test [14].

RESULTS

A total of 445 NFGNB were isolated from respiratory samples. Endotracheal secretions

were the commonest sample followed by bronchoalveolar lavage and sputum *A. baumannii* was the most common isolate (58.8%), followed by *P. aeruginosa* (34.6%). Other nonfermenters isolated were *P. stutzeri, B. cepacia* (BCC), *S. maltophilia, Sphingobacterium, A. lwoffii,* and *A. haemolyticus*. (Figure -1) *P. aeruginosa* isolates showed lesser resistance to AMA as compared to *A.baumannii*. 40% of *A.baumannnii* and 20% of *P.aeruginosa* isolates showed resistance to imipenem. (Figure-2) *P.stutzeri* showed 100% susceptibility to piperacillin, piperacillin/tazobactam, imipenem. *BCC* and *S.maltophilia* showed good activity for cotrimoxazole. None of the other non fermenters showed resistance to imipenem. Overall resistance to imipenem was 30.5% and MBL production was detected in 80.1% of NFGNB isolates. 83.8% of *P.aeruginosa* & 79.8% of *A. baumannii* showed MBL production.(Figure-3) MBL positive isolates of *A. baumannii and P. aeruginosa* were significantly resistant to aminoglycosides, ciprofloxacin, cephalosporins, piperacillin, piperacillin-tazobactam (P<0.05) as compared to MBL negative isolates.

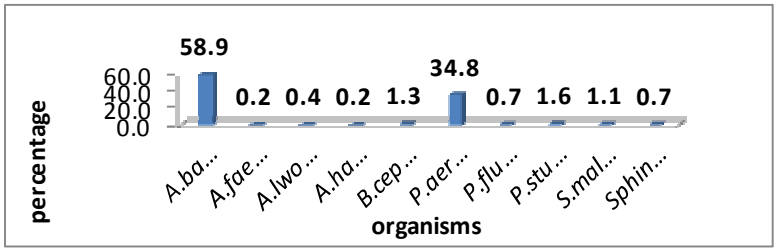

Figure 1
Frequency of NFGNB (n= 445) in respiratory samples

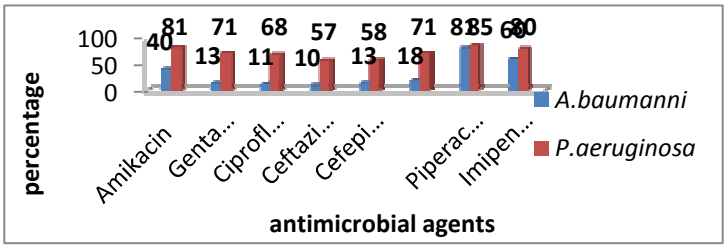

Figure 2
Antimicrobial sensitivity profile of common NFGNB isolates

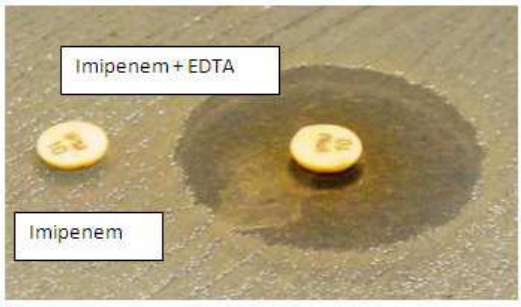

Figure 3
Imipenem EDTA combined disc test (MBL positive isolate)

DISCUSSION

Non fermenting gram-negative bacilli that were considered to be contaminants in the past have now emerged as important healthcare-associated pathogens. *A. baumannii* and *P. aeruginosa* were common respiratory isolates. Piperacillin/tazobactam and imipenem were the most effective antibiotics for NFGNB. Cotrimoxazole showed good activity for *BCC* and *S.maltophilia* isolates. Similar spectrum was reported in literature[15]. Resistance to imipenem was observed in 40% of *A .baumannii* and 20% of *P. aeruginosa* isolates in our study whereas 42.8 %[16] and 21 % [17] of *P. aeruginosa* and 14.2 %[16] and 23 % [17] of *Acinetobacter* spp were resistant to imipenem as reported in literature . In our study resistance to carbapenems was observed in 30.5% isolates whereas varying rates of resistance to carbapenems 12%[18],12.2%[16],36.4%[9] was reported in literature. However 94% of *P. aeruginosa* and *100% of Acinetobacter* spp isolates were sensitive to imipenem in a study by Malini *et al*[15]. Among imipenem resistant isolates, 83.8% of *P. aeruginosa* & 79.8% of *A. baumannii* showed MBL production whereas higher MBL production in *Acinetobacter spp*. (96.6%) and *P. aeruginosa* (100%) and lower MBL production in *Acinetobacter spp*. (56%) and *P.aeruginosa* (50%) as compared to our study was reported in literature [17, 19]. A high percentage of MBL production (80.1%) was observed in our study consistent with reports where MBL production varied from 85.7% - 100%[19, 20]. Majority of MBL positive isolates showed resistance to third generation cephalosporins, aminoglycosides and quinolones which is consistent with various studies [21, 22]. Production of MBL has tremendous therapeutic consequences since these organisms also carry multidrug resistance genes and the only viable option remains the potentially toxic polymyxin B and colistin [23].

CONCLUSION

To conclude, emergence of MBLs producing NFGNB in our clinical strains is alarming therefore regular monitoring and documentation of carbapenem resistance is crucial. 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.Stringent protocols such as antibiotic policies and resistance surveillance programs are mandatory to curb these bacteria in ICU settings.

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