Original Article

Prevalence and Anti-biogram of Carbapenem Resistant Gram-negative Bacteria with Phenotypic Detection of Metallo-beta-lactamase

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Background : Development of Carbapenem Resistance is on the rise in many countries including India. The risk factors for development of carbapenem resistance include poor living conditions, easy over the counter availability of antibiotics, over- or under dosage of antibiotics or presence of poor-quality drugs in the market. Though there are many mechanisms by which carbapenems develop resistance, resistance due to Metallo-beta-lactamase (MBL) is critical as being plasmid mediated, it can rapidly spread to the environment.

Aims and objectives : To determine the prevalence and antibiotic susceptibility pattern of Carbapenem Resistant Gram Negative Bacteria (CRGNB) with phenotypic detection of MBL

Materials and Methods : All the samples received in microbiology lab were processed by standard methods and Carbapenem Resistant Gram Negative Bacilli (CRGNB) were identified by automated system. Imipenem resistant isolates were subjected to detection of MBL by Combined Disk Synergy Test (CDST) & Epsilometer test (E test).

Results : The prevalence of CRGNB was found to be 21.4% in our study. Majority of the isolates were *Klebsiella pneumoniae* (41.7%) followed by *E coli* (23.3%) and *Acinetobacter baumanni* (15.9%). In 84% isolates were MBL producers by E test and 83% were MBL producers by CDST.

Conclusion : To control the spread of MBL, active surveillance in the microbiology lab along with strict infection control practices and antibiotic stewardship program needs to be set up in each health care setting.

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Key words **: Carbapenem Resistance, CRGNB, Imipenem, MBL.**

Antibiotic resistance has emerged as a global burden and a threat to the public health over the past few years. β-lactam antibiotics are the most common group of antimicrobials used for the treatment of majority of the Gram Negative Bacterial (GNB) infections[1]. Along with cephalosporins and penicillin, the carbapenems have become an important therapeutic option for Intensive Care Unit patients. They are used as a last resort against many multi drug resistant, Gram negative bacteria, especially in cases of infections due to Extended Spectrum Beta Lactamase (ESBL) and Amp C beta lactamase (AmpC) enzyme producing *Enterobacteriaceae*[2]. There has been an increase in the emergence of carbapenemases like metallo β lactamase (MBL) as a result of frequent use of carbapenems. MBL can hydrolyze a wide variety of β lactam agents, such as penicillins, cephalosporins, and carbapenems. MBLs are inhibited by thiol based compounds and metal chelators, such as an Ethylene Diamine Tetra Acetic Acid (EDTA)[3,4].

Editor's Comment :

- Carbapenem resistant isolates are on a high in India.
- Timely detection of carbapenem resistant isolates is crucial to initiate appropriate and targetted therapy.
- Strict antimicrobial stewardship practices also need to be implemented in every health care setting to reduce the incidence of carbapenem resistant Gram negative bacteria in our health care settings.

Many GNB, such as *Acinetobacter species*, *Pseudomonas spp*, some of the *Enterobacteriaceae* can easily produce MBL. MBLs can be transferred through plasmids and can cause outbreaks and nosocomial infections[4]. This can mainly affect the patients in Intensive Care Units (ICU's) who have many co-morbidities or patients who are on prolonged antibiotics[1]. Therefore, identification and early detection of MBL is essential to provide prompt treatment to the patient which can help in reducing morbidity and mortality due to carbapenemase producing GNB.

Materials and Methods

A total of 163 CR Gram Negative Bacilli were included in the study (Calculated from a former similar

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study)[3]. All these isolates were subjected to antibiotic susceptibility testing as per Clinical & Laboratory Standards Institute (CLSI) guidelines using Vitek 2 automated system (BioMerieux, Marcy l'Etoile, France)[5]. Imipenem (IPM) resistant strains were taken as positive for Metallo-beta-lactamase enzyme (MBL) screening. The isolates which gave positive MBL screening test were confirmed by using two methods with inhibitor of MBL, Ethylene Diamine Tetra Acetic Acid (EDTA). By dissolving 18.61 g of EDTA (Hi Media Laboratories Pvt Ltd, India) in 100 ml of distilled water 0.5 M EDTA was prepared[4].

Combined Disk Synergy Test (CDST) with 0.5 M EDTA[4] :

Two IPM (10 µg) disks (Hi Media Laboratories Pvt Ltd, India) were placed 30 mm apart from center to center on the surface of a Muller Hinton agar plate (MHA) (HiMedia Laboratories Pvt Ltd, India) and 10 µl 0.5 M EDTA solution was applied to one of the disks to obtain the required concentration of 750 µg. If zone of inhibition of IPM-EDTA disk was ≥7 mm more than that of IPM disk alone, it was considered as MBL positive[4].

E-test[6] :

An E-test MBL strip (Hi Media Laboratories Pvt Ltd, India) contain a double-sided seven-dilution range of IPM (4-256 µg/ml) and IPM (1-64 µg/ml) in combination with EDTA. On MHA (Hi Media Laboratories Pvt Ltd, India), a lawn culture of 0.5 McFarland opacity standard of the test isolate was done after which the E-strip was placed. The plates were observed for IPM and IPM-EDTA Minimum Inhibitory Concentration (MIC) values after overnight incubation. If the MIC ratio of IPM/IPM plus EDTA was more than eight, the test was considered positive[6].

Results

A total of 163 Carbapenem Resistant Gram Negative Bacilli were processed during the study period. Age-wise distribution of the isolates are given in Table 1. 62% of the isolates were obtained from male patients while only 32% were from female patients. 37% of the patients were admitted to the ICU's (Table 2). 34% of the isolates were obtained from urine sample, 19% from pus, 17 % from blood and rest were isolated from respiratory sample like sputum, Bronchoalveolar Lavage (BAL) and Endotracheal Tube Aspirate (ETT) (Fig 1). 41% of the isolates were *Klebsiella pneumoniae*, 23% were *E coli* and 16% were *Acinetobacter baumanni* (Table 3). A total of 136 isolates were MBL producers by Combined Disc Synergy Test (CDST) while 138 isolates were MBL producers by E test (Fig 2). 96% of the isolates were susceptible to tigecycline, 84% were susceptible to fosfomycin and 92% to colistin (Fig 3).

Table 1 — *Age wise distribution of patients*

Age group (in years)	Number of patients
0-1	11
2-10	4
11-20	10
21-30	9
31-40	8
41-50	24
51-60	16
61-70	28
71-80	11

Table 2 — *Location of the patients*

Location of the patients	Number of patients n (%)
Medicine	23 (14%)
Surgery	9 (5%)
Orthopedics	15 (9%)
Pediatrics	2 (1%)
ICU	43 (26%)
NICU & PICU	18 (11%)

ICU - Intensive Care Unit, NICU - Neonatal ICU, PICU - Paediatric ICU

Table 3 — *Organism isolated*

Gram Negative Bacilli isolated	Number of patients
E coli	38
Klebsiella pneumoniae	68
Enterobacter cloacae	5
Enterobacter aerogenes	2
Proteus mirabilis	1
Pseudomonas aeruginosa	8
Acinetobacter baumanii	26
Burkholderia cepaciae	2
Elizabethkingia meningoseptica	1
Sphingobacterium spiritivorum	1
Chryseobacterium indologenes	2
Alcaligens faecalis	2

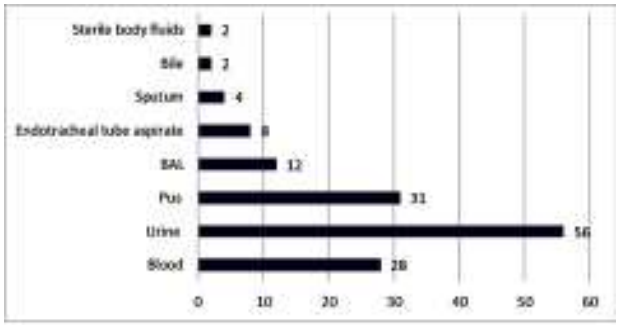

Fig 1 — Sample-wise distribution of patients

Fig 2 — MBL producer by CDST and E test

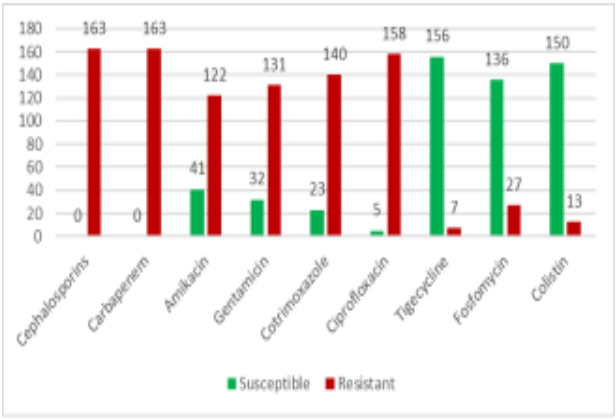

Fig 3 — Antibiotic susceptibility pattern of isolates

Discussion

Out of the 760 Gram Negative Bacilli obtained during the study period, 163 isolates were Carbapenem Resistant. The prevalence of Carbapenem Resistant Gram Negative Bacilli (CRGNB) in our hospital is 21.4%. The prevalence of CRGNB was 12% in a study by *Nair PK, et al* in western India (2013), 11% in a study by *Ralte VSC, et al* in eastern India (2022), 12% in study by *Gladstone P, et al* in southern India (2005) and 11% in a study by *Garg A, et al* in Northern India (2019)[2,7-9]. The high prevalence of CRGNB in our study could be due to the fact that majority of the patients were admitted in Intensive Care Units where they were already exposed to antibiotics possibly for more than 4 weeks. Also, most of these patients would require mechanical ventilation, urinary catheter or central line thus increasing the chance of getting infection with resistant bugs[10]. In 48% of the patients were above 40 years of age. Similar age distribution was seen in study by *Abhishek S, et al*[6]. Reason for risk of CRGNB above this age could be due to weaker immune system and presence of co-morbidities[11]. In study by *Gao Y, et al* chance of 60-day survival in patients above 55 years with bloodstream infection caused by CRGNB was 42% and chance of 60-day death was 63%[12]. 105 patients were males (62%) and 58 were females. Males have higher prevalence of developing Gram negative bacterial infection.[13] In a study by *Gomila A, et al* one of the predictors for development of infection caused by multidrug resistant bacteria was male gender[14]. Study by *Satyajeet K Pawar, et al* in Maharashtra also showed male predominance (65.3%)[15]. Sample-wise distribution of the isolates showed that majority were obtained from urine (34%; 56/163) followed by pus, blood and respiratory specimens. Other predictors for development of infection caused by multidrug resistant bacteria were the presence of Urinary Tract Infection and development of UTI during hospital stay in the study by *Gomila A, et al*[14]. 41% of the carbapenem resistant isolates were *Klebsiella pneumoniae* (68/163). Highest rate of Carbapenem Resistance (CR) is seen among *Klebsiella pneumoniae* as compared to other *Entero-bacteriaceae* globally. In Italy, CR rate among *Klebsiella pneumoniae* is 33%, 62% in Greece,37% in Saudi Arabia and 11% in the United states[13,16]. In India, *Klebsiella pneumoniae* was the predominant CRGNB (44%) in various studies like the study by *Porwal R, et al* (44%), *Abhishek S, et al* (55%) and *Satyajeet K Pawar, et al* (63%)[10,11,15]. In the US, *Klebsiella Pneumoniae* Carbapenemase (KPC) is the most frequently produced carbapenemase by CR *Enterobacteriaceae* but in India, MBL is the most commonly produced Carbapenemase[16]. In 84% of the CRGNB in the present study were found to be MBL producers by E-test and 83% by CDST. In a similar study of detection of MBL by *Panchal CA, et al* 70% of the CRGNB were MBL producers[4].

CRGNB are usually resistant to all beta lactam antibiotics, beta lactam-beta lactamase inhibitors (except ceftazidime-avibactam) and Carbapenems. They are usually susceptible to tigecycline and polymyxins (colistin and polymyxin B)[16-19]. In the present study also all the isolates were 100% resistant to first, second and third generation of cephalosporins, piperacillin-tazobactam, cefoperazone-sulbactam, ertapenem, imipenem and meropenem. 96% (158/163)of the isolates were susceptible to tigecycline, 84% (136/163) were susceptible to fosfomycin and 92% (150/163) to colistin. Typically, NDM producers are resistant to all aminoglycosides[16,20]. 75% of the isolates were resistant to amikacin, 80% to gentamicin, 85% to cotrimoxazole and 97% to ciprofloxacin in our study.

Conclusion

The first step in dealing with the problem of MBL is the identification of infected patients. Active surveillance for the most MBL needs to be done in the microbiology laboratory. To initiate appropriate and targeted therapy and to reduce the chance of development of antibiotic resistance, timely detection of MBL is crucial. This is because, MBL infected/colonised patients can serve as a reservoir of infection thus contaminating the environment. To prevent this, such patients need to be contact isolated and vigilant infection control practices like hand hygiene, proper waste disposal should be maintained. Although

molecular techniques are regarded as the gold standard for detection of MBL, in routine diagnostic laboratory, it becomes impractical due to its requirement of costly infrastructure and trained personnel. Rapid and effective phenotypic detection of MBL is therefore the need of the hour. From our study, CDST and E test was found to be almost equally efficient for detection of MBL. To control Carbapenem Resistance, strict antimicrobial stewardship practices also need to be implemented in every health care setting. Limiting the use of invasive procedures can also contribute towards prevention of Carbapenem Resistance in hospitals.

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