CARBAPENEM RESISTANCE IN CLINICALLY SIGNIFICANT NON FERMENTING GRAM NEGATIVE BACILLI

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ABSTRACT: BACKGROUND/OBJECTIVES: The growing increase in the rates of antibiotic resistance is a major cause for concern in both non-fermenting bacilli. β -lactams have been the mainstay of treatment for serious infections, and the most active of these are the carbapenems. 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. We have undertaken this study to ascertain the prevalence of MBL-producing non-fermenting bacilli. **MATERIALS AND METHODS:** The study included 93 isolates of NFGNB from Blood, endotracheal aspirates, sputum, urine, urinary catheter tip & pus between January 2012- January 2013. Antibiotic susceptibility to antibiotics was done by Modified Kirby Bauer method. All the isolates with a zone size ≤ 19 mm for imipenem were included in our study to detect the presence of MBL by Imipenem-EDTA double-disc synergy test (DDST). Imipenem resistant, non MBL isolates were tested by Modified Hodge test & Amp C β lactamases. **RESULTS:** Of the 93 imipenem resistant NFGNB, 27(29.03%) were *Pseudomonas* spp., 66(70.96%) were *Acinetobacter* spp. Among them, 10(37.03%) *Pseudomonas* spp. and seven (10.60%) *Acinetobacter* spp. were MBL positive by Imipenem EDTA double-disc synergy test (DDST). Five (8.40%) *Acinetobacter* spp. were Amp C positive and one (5.88%) strain of *Pseudomonas* showed positive Modified hodge test. **CONCLUSION:** MBL production is an important mechanism of carbapenem resistance among *Pseudomonas* spp. but not among *Acinetobacter* spp. Carbapenemases other than MBL may be responsible for carbapenem resistance in NFGNB.

KEY WORDS: Non fermenting gram negative bacilli, imipenem, metallo- β -lactamases

INTRODUCTION: The introduction of carbapenems into clinical practice represented a great advance for the treatment of serious bacterial infections caused by beta-lactam-resistant bacteria. Due to their broad spectrum of activities and stability to hydrolysis by most of the beta-lactamases, the carbapenems have been the drug of choice for the treatment of infections caused by penicillin or cephalosporin-resistant Gram-negative bacilli, especially ESBL Gram-negative infections.¹ However, carbapenem resistance has been observed frequently in non-fermenting bacilli *Pseudomonas aeruginosa* and *Acinetobacter* spp.² Resistance to carbapenem is due to decreased outer membrane permeability, increased efflux systems, alteration of penicillin-binding proteins and carbapenem hydrolyzing enzymes-carbapenemase, increased Amp C β lactamase production.

Carbapenemases are class B metallo- β -lactamases (MBLs; IMP, VIM) or class D oxacillinases (OXA 23 to OXA 27) or class A clavulanic acid inhibitory enzymes (SME, NMC, IMI, KPC).³ Metallo- β -lactamase belongs to class B, which requires divalent cations of zinc as cofactors for enzyme activity. They have potent hydrolyzing activity not only against carbapenem but also against other β -lactam antibiotics.

The IMP and VIM genes responsible for MBL production are horizontally transferable via plasmids and can rapidly spread to other bacteria. Modified Hodge test is a phenotypic method for detection of carbapenemases.⁵ The double disk synergy test has been modified using EDTA instead of a β -lactamase inhibitor. This test detects and differentiates carbapenemase and metallo- β -lactamase production from other β -lactamases.⁶ This study focuses on the detection of carbapenemase and metallo- β -lactamase production in imipenem resistant clinical isolates of NFGNB.

AIM AND OBJECTIVES:

- To identify, characterize and determine the antibiogram of non fermenting gram negative bacilli isolates from clinical samples.
- To determine the prevalence of carbapenemase and metallo beta lactamase producing non fermenting gram negative bacilli in this locality by phenotypic methods.

MATERIALS AND METHODS: The cross sectional study was conducted in the Department of Microbiology, Kasturba Medical College and hospital, Mangalore for a period of one year January 2012-January 2013.

Specimens: The study included 93 isolates of NFGNB from Blood, endotracheal aspirates, sputum, urine, urinary catheter tip & pus.

Detailed methodology:

Isolation and identification: The specimens were processed by standard protocols and the non fermenting Gram negative bacilli were identified by standard procedures and Vitek 2 Compact system.⁷

Antimicrobial susceptibility testing: All the isolates were subjected to antibiotic susceptibility testing by Kirby Bauer disk diffusion method using the following antibiotics: Amikacin (30 μ g), ceftazidime (30 μ g), cefaperazone /sulbactam (30 μ g), Cefepime (30 μ g), ceftriaxone (30 μ g), Cotrimoxazole (23 μ g). Imipenem (10 μ g), Piperacillin (100 μ g), Ticarcillin/clavulanic acid (75/10 μ g), Ciprofloxacin (5 μ g),⁸

Control strains included: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853.

All the isolates with a zone size ≤ 19 mm for imipenem were included in our study to detect the presence of MBL by Imipenem-EDTA double-disc synergy test (DDST).⁹

Imipenem resistant , non MBL isolates were tested by Modified Hodge test & Amp C β lactamases.^{10,11}

RESULTS:

- Of the 93 imipenem resistant NFGNB, 27(29.03%) were *Pseudomonas* spp. ,66(70.96%) were *Acinetobacter* spp.
- Among them, 10(37.03%) *Pseudomonas* spp. and seven (10.60%) *Acinetobacter* spp. were MBL positive by Imipenem EDTA double-disc synergy test (DDST).
- Five (8.40%) *Acinetobacter* spp. were AmpC positive and one (5.88%) strain of *Pseudomonas* showed positive Modified hodge test.

DISCUSSION: Carbapenems have a broad spectrum of antibacterial activity, and these are resistant to hydrolysis by most β -lactamases including extended spectrum β -lactamases (ESBL) and AmpC β -lactamases. These are often used as a last resort in infections due to multidrug resistant Gram-negative bacilli. There has been an increase in reports of carbapenem resistance in *Acinetobacter* species and *P. aeruginosa*.

The most important mechanism of carbapenem resistance was MBL production among *Pseudomonas* species. Various methods have been recommended for screening MBL. These include the modified Hodge test, double-disc synergy test using imipenem and EDTA discs or ceftazidime and EDTA discs, EDTA-impregnated imipenem discs and EDTA-impregnated meropenem discs. EDTA disk synergy test is more sensitive for detecting carbapenemases. Carbapenem resistance is an emerging problem among NFGNB. The rate of MBL production in *Pseudomonas* spp. (37.03%) and *Acinetobacter* spp. (10.06%) in concordance with other studies. According to the previous studies, MBL production ranged from 7 to 65%.^{12,13}

Amp C beta lactamases is found in (8.47%) of the isolates of *Acinetobacter* spp. which could be important factor leading to carbapenem resistance could be an important contributory factor for meropenem resistance among the isolates in our hospital similar to other studies. Amp C β -lactamases were found in 42.85% of the resistant isolates and may contribute to the carbapenem resistance in these isolates.¹⁴

The detection of MBL and other carbapenemases is of utmost importance in deciding the most appropriate therapeutic regimen for treatment of infections.

CONCLUSION:

- MBL production is an important mechanism of carbapenem resistance among *Pseudomonas* spp. but not among *Acinetobacter* spp.
- Carbapenemases other than MBL may be responsible for carbapenem resistance in NFGNB

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