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CARBAPENEMS RESISTANCE IN GRAM-NEGATIVE BACILLI ISOLATES FROM RESPIRATORY TRACT SAMPLES OF PATIENTS IN INTENSIVE CARE UNITS

Vandanan B. Rajput* **and Dr. Jigna P. Naik**

Arts, Science & Commerce College, Department of Microbiology (PGDMLT), Kholwad, Surat, Gujarat, India.

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***Correspondence for Author**
Vandanan B. Rajput
Arts, Science & Commerce College, Department of Microbiology (PGDMLT), Kholwad, Surat, Gujarat, India.

ABSTRACT

Carbapenems are preferred antibiotics for severe infections in ICUs. Its widest antibacterial spectra offers realistic option for monotherapy in ICUs, but in recent year clinical utility of carbapenems are under a serious threat, due to production of Metallo-beta-Lactamase (MBLs). Gram-negative bacterial infections are particular concern, especially MBLs producing in intensive care units (ICUs). Detection of MBLs among Gram-negative bacilli (GNBs) is crucial for the optimal treatment of patients and to control the spread of resistance. This study was conducted to determine carbapenems-resistant and the proportion of MBLs producer among multi-drug resistant (MDR) GNBs isolated from respiratory tract samples of patients admitted in various ICUs.

Total 70 non-duplicate GNBs from respiratory tract samples were isolated, identified and subjected to antibiotics susceptibility testing. Isolates were screened for MBLs production and confirmed by phenotypic confirmatory tests. Out of 70 GNBs, 38 isolates were found to be MDR, 31 isolates were found to be imipenem resistant, 34 isolates were found to be meropenem, ertapenem and doripenem resistant. These 34 carbapenems resistant isolates were found MBLs producer by phenotypic screening and confirmatory test, with broad spectrum resistance profile.The results suggested that the resistance to carbapenems agents in the present study is due MBLs production. Considering the emergence characteristic of MBLs, its surveillance becomes important to control the transmission of this resistance mechanism.

KEYWORDS: Intensive Care Units (ICUs), Respiratory tract sample, Gram negative bacilli (GNBs), Carbapenems resistance, Metallo-Beta-Lactamase (MBLs).

1. INTRODUCTION

Carbapenems are beta lactam antibiotics, presently considered as the most potent agents for treatment of infections caused by multidrug resistant (MDR) Gram-negative bacterias (GNBs), due to its stability, against the majority of beta lactamases and their high rate of permeation through bacterial outer membranes. However, there have been reports of resistance to carbapenems.[1] This is of great concern as presently to combat infections by MDR-GNBs, carbapenems are considered the last resort especially in intensive care units (ICUs) and high risk wards.

Infections in ICUs are a source of great concern globally because of their impact on patient morbidity and mortality as well as their impact on the cost of patient care. One important feature of these infections which continues to pose a therapeutic challenge is the increasing resistance to multiple antibiotics. It is internationally accepted that the most resistant organisms often appear first in the ICUs, where patients are debilitated, very often have multiple lines and tubes and have been exposed to a wide array of antibiotics. The driving forces for antimicrobial resistance include poor infection control practices and the overuse/misuse of antibiotics.[2] The emergence of organisms that are resistant to all the antibiotics usually used against them (pan-resistant) is alarming. This situation becomes even grimmer because relatively few new antibiotics with activity against GNBs are being developed.

Respiratory tract infection (RTI) is a major cause of death in developing countries. RTI are the most common hospital-acquired GNBs infections, about 30% health acquired infection affects ICUs patients so treatment is in compromised.[3] Acute respiratory infection (ARI) is the leading cause of morbidity and mortality in critically ill patients and MDR-GNBs form a major problem in ICU's.[4]

Clinical utility of carbapenems are under a serious threat with the emergence of acquired carbapenemases resistance mechanisms, mainly; class B Metallo-β-lactamases (MBLs).[5]

MBLs confer high-level resistance to all β-lactams except aztreonam and susceptible against metal chelators.[6]

They are of significant concern because they restrict the therapeutic options, cause treatment failures and are increasing in occurrence worldwide. These enzymes are associated with the potentially fatal laboratory reports of a false susceptibility to the cephalosporins and carbapenems, that can lead to the prescription of the inappropriate therapy for the infected patients.[7] The detection of the MBLs mediated resistance in the clinical microbiology laboratory poses a problem, because the phenotypic tests are not standardized. The Clinical Laboratory Standards Institute (CLSI) has not yet published the guidelines for their detection.

The need to use the available antibiotics wisely, in order to maximize their impact and prolong their usefulness, cannot be overemphasized. It is therefore important to know the local antibiotic resistance patterns as these may differ from other settings and is required to inform appropriate local antibiotic use. In order to control the spread of resistant bacteria, local surveillance data should play an integral role in developing effective intervention strategies.[8]

Keeping this background, the goal of this study was to determine carbapenems-resistant and the proportion of MBLs producer among MDR-GNBs isolated from respiratory tract samples of patients admitted in ICU's.

2. MATERIALS AND METHODS

Study period and clinical samples: This study was conducted in tertiary care hospital at Surat, "between" December 2013 to May 2014. A total of 70 respiratory tract (RT) specimens [Endotracheal secretion (ET, 44), Bronchoalveolar lavage (BAL, 04) and sputum (22)] were collected with universal safety precautions,[9] from ICUs for culture and sensitivity.

Bacterial Identification: All the specimens were inoculated on Chocolate agar (CHA), 5% Sheep Blood agar (BA) and MacConkeys agar (MA), plates were incubated at aerobically 37^0C for 18-24 hours. The isolates were then identified by standard and conventional microbiological techniques.[9,10]

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing was performed by Kirby-Bauer disk diffusion method[11] and results were interpreted as per CLSI- M100-S21, 2011 recommendations.[12] Multidrug resistance was defined as resistance to three or more of the first line antimicrobial agents belonging to different structural classes.[13] Following

antibiotic disk were used for antibiotic susceptibility test, Ciprofloxacin 5µg/disk (CIP), Ofloxacin 5µg/disk (OF), Levofloxacin 5µg/disk (LE), Amikacin 30µg/disk (AK), Gentamicin 30µg/disk (GEN), Netilmicin 30µg/disk (NET), Tobramycin 10µg/disk(TOB), Ceftazidime 30µg/disk (CAZ), Ceftriaxone 30µg/disk (CTR), Cefotaxime 30µg/disk (CTX), Cefepime 30µg/disk (CPM), Imipenem 10µg/disk (IPM), Meropenem 10µg/disk (MRP), Ertapenem 10µg/disk (ETP), Aztreonam 30µg/disk (AT), Piperacillin 100µg/disk (PI), Piperacillin-tazobactam 100/10µg/disk (PIT), Chloramphenicol 30µg/disk (C), Polymyxin B 300U/disk (PB) and Colistin 10µg/disk(CL). For quality control *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 strains were used.

Detection of MBLs

MBLs screening method: Screening was carried out by Kirby Bauer disk diffusion method as per CLSI guidelines.[14] If one carbapenem i.e. Imipenem resistant phenotypes were considered, then hidden MBLs carrying isolates would be missed.[15] Isolates resistant to Imipenem, Meropenem, Ertapenem and Ceftazidime were considered as screening positive

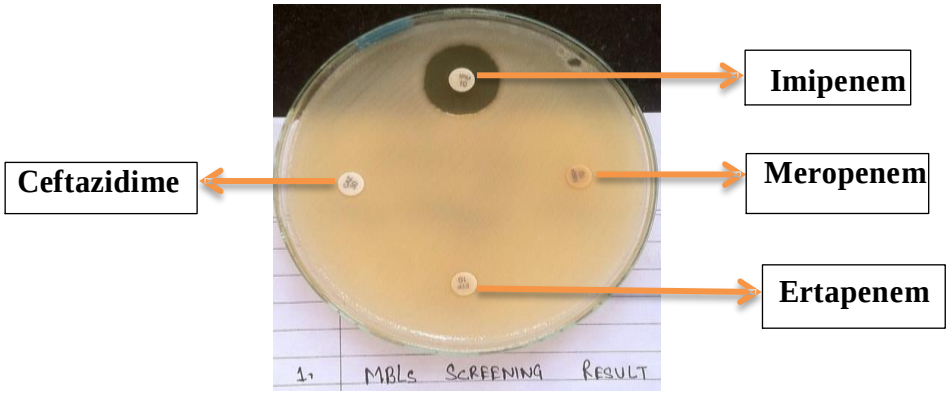


Figure 1.MBLs Screening Test Result

MBLs Confirmation test: All screening positive isolates were subjected to, phenotypic confirmatory tests.

1. EDTA Disk Synergy (EDS) Test[16]: In EDS test, 0.5 M EDTA solution was prepared by dissolving 18.61 g. EDTA (Hi-Media, India) in 100mL of distilled water and adjusting its pH 8 by using NaOH and was sterilized by autoclave. An overnight liquid culture of the test strain were adjusted to a turbidity of 0.5 McFarland standard and spread on the surface of Mueller-Hinton agar plate. After drying, 10µg/disk imipenem disk and blank filter paper (Whatmann filter paper no. 1, 6mm in diameter) disk were placed 10 mm apart from edge to

edge, and 10µL of 0.5 M EDTA solution was then applied to the blank disk. In addition to imipenem, three different beta-lactams: meropenem, ertapenem & ceftazidime were used with EDS test, for MBLs detection and named as modified-EDS (m-EDS) test. After incubating overnight at 37^0C, the strain shows a synergistic zone of inhibition between imipenem, meropenem, ertapenem & ceftazidime disks with EDTA disks were interpreted as MBLs positive as shown in Fig: 2 A, B & C.

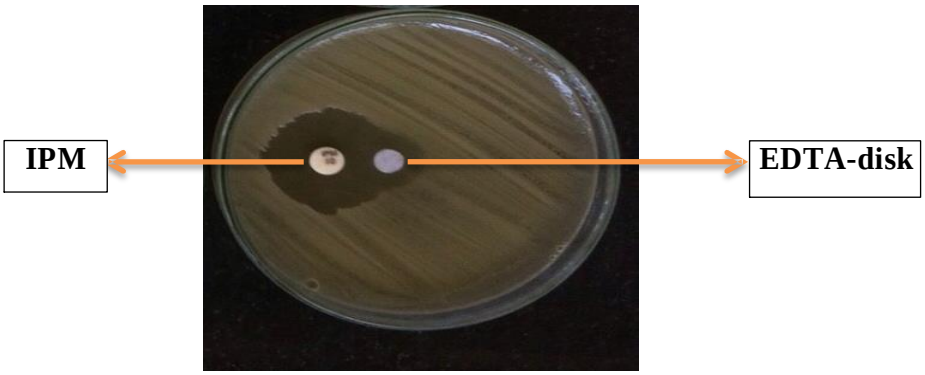


Figure 2: (A) EDTA Disk Synergy Test:Positive strains showing a synergistic zone of inhibiton between Imipenem (IPM) with EDTA-disk.

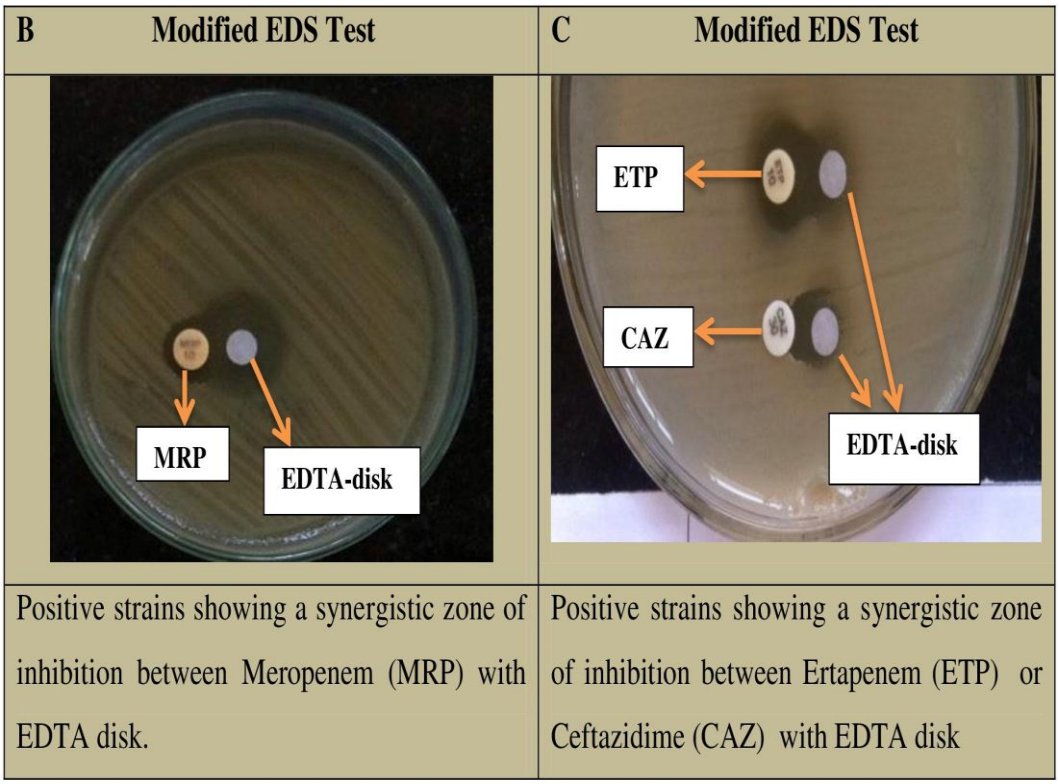


Figure 2. (B) & (C) Modified EDTA Disk Synergy Test.

2. E-test[17]: The Meropenem E-test MBLs detection strips (Himedia, Mumbai, India) consists of Meropenem (MRP) (4-256 µg/ml) and Meropenem + EDTA (1-64 µg/ml) were used. An overnight liquid culture of the test strains were adjusted to a turbidity of 0.5 spread on the surface of Mueller-Hinton agar plate. After drying the strip was placed onto the inoculated agar surface. The plates were incubated at 37^0C for 18 to 24 hours. The MIC values were read where the respective inhibition ellipses intersected the strip in accordance with the manufacturer's instructions. When the ratio of the value obtained for Meropenem: the value of Meropenem+EDTA is >8, interpreted as MBLs positive,as showen in Fig: 3.

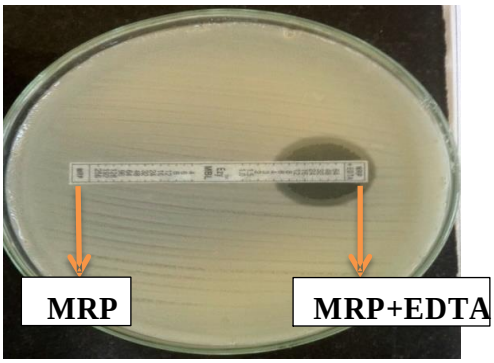


Figure 3. Meropenem E-Test: MBLs positive stains showing ratio > 8 µg/ml.

3. RESULT & DISCUSSION

Of the 70 non-duplicate GNBs, the maximum number of strains isolated were *Klebsiella spp.* (28) followed by *Pseudomonas aeruginosa* (21), *Acinetobacter spp.* (10), *Escherichia coli* (10), & *Burkholderiacepecia complex* (01)**.**

Antibiotics susceptibility test result: We detected 38 (54.2%) as MDR.

Screening test result: Out of 38 MDR strains, 31 isolates were found to be imipenem resistant, and 34 isolates were found to be meropenem, ertapenem and doripenem resistant, were considered as screening positive for MBLs.

Phenotypic confirmatory test result: Among the 34 screening positive isolates 31 isolates were confirmed MBLs producer by EDS test. Remaining 3 imipenem sensitive strains were MBLs producer by m-EDS test. All 34 isolates were also confirmed by E-test.

Of 34 MBLs producing GNBs, the *Pseudomonas spp.*(11) was the predominant organism, as carbapenem resistance *Pseudomonas spp.* infection in ICUs has been frequently reported by several authors, which are associated with higher mortality,[18] followed by *Klebsiella*

spp.(10) *Acinetobacter spp.*, (8) & *Escherichia coli*(5) as shown in Fig: 4, in contrast to study of Kombade & Agrawal., 2014 in ICUs, maximum number of isolates were *Acinetobacter spp*. (13), *Pseudomonas spp.* (4), *Klebsiella spp.* (3) and *Escherichia coli* (0).[19]

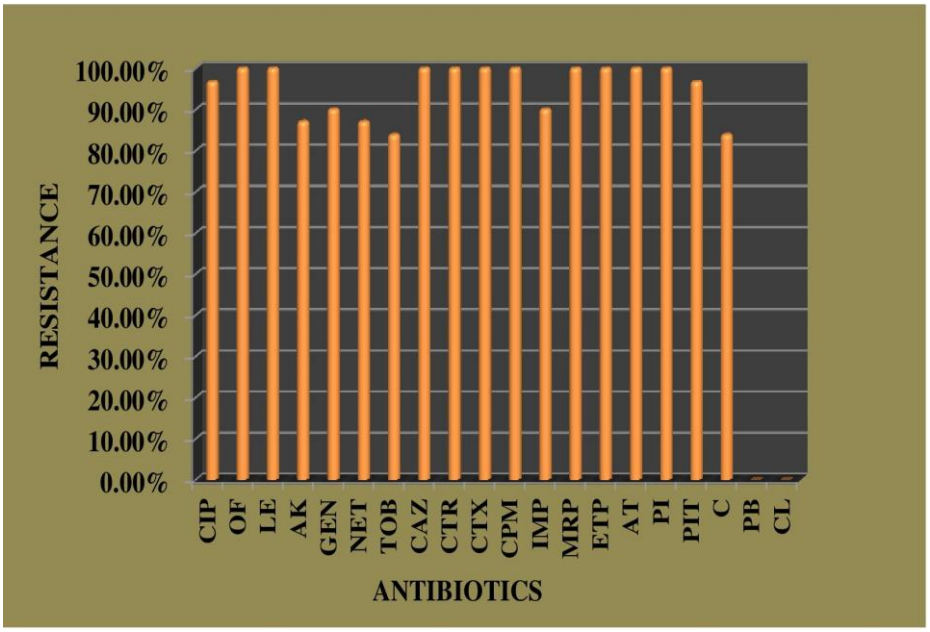
This study documents, 48.57% carbapenems resistance GNBs. It shows a considerably higher prevalence of resistance. Reportedly, several outbreaks due to carbapenems resistant GNBs have resulted with considerable morbidity and mortality. Resistance to these antibiotics is due to decreased outer membrane permeability, increased efflux systems, alteration of penicillin binding proteins and carbapenems hydrolyzing enzymes – carbapenemases.[20]

Carbapenemases may be defined as B-lactamases that significantly hydrolyze at least imipenem or/and meropenem, in which class B enzymes are the most clinically significant carbapenemases, they are metallo-enzymeses-MBLs. They have been reported worldwide but mostly from South East Asia and Europe.[21]

The infections which are caused by carbapenems-resistant GNBs that produce various β-lactamase enzymes have been reported with an increasing frequency in ICUs. In our study, all carbapenems resistant isolates were also MBLs producers 48.57%, while in Kombade & Agrawal., 2014 in ICUs, they found 18.8%, which is very low as compare to our study.[19]

In addition among the all 34 MBLs in GNBs, predominant source of MBLs producer was ET specimen i.e.27, which may correlates the use of indwelling medical devices are common in these areas, which can play an important role in the spread of infective agents, very often associated with high levels of morbimortality.[22]

Antibiotics susceptibility of MBLs producers showed 100% sensitive to toxic peptide antibiotics polymyxin B & colistin. These antibiotics are associated with high incidence of nephrotoxicity and neurotoxicity which limits their uses.[23] In contrast, aztreonam susceptibility was common feature of MBLs producing organisms, but in our study all 34 strains were 100% resistance, which may showed other co-existing resistance mechanism.[24] Antibiotics resistance profile of carbapenems resistance MBLs producing isolates were as shown in (Graph no.1).



Graph 1: Antibiogram of Carbapenems resistance MBLs producing isolates in ICUs.

Since carbapenem resistance is mediated by several mechanisms, cross resistance is commonly seen among related antibiotics. Although there are various specific tests[25, 26] to detect the underlying mechanism of carbapenem resistance, Kirby-Bauer disc diffusion test is a simple, easy to perform and cost effective test which can be conveniently used to screen carbapenem resistance. These strains also remain resistant to several other antibiotics including penicillins, cephalosporins, quinolones, amino glycosides and third generation cephalosporins including ceftazidime and cefotaxime.[20] In this study, all strains were resistant to all antibiotics tested so that there was no alternative antibiotic that could be used to treat infections caused by them. This poses serious problems in choosing the right antibiotic for the treatment of sick patients admitted into the ICUs.

4. CONCLUSION

In our study, we found high level of resistance against beta-lactam group of antibiotics including carbapenems; it may correlate to excessive use of broad-spectrum antibiotics. This study emphasizes that carbapenems resistant organisms are increasingly worldwide. Enzymes that may be clinically worrying are of MBLs. These enzymes confer resistance to carbapenems, expressed either a naturally occurring or an acquired. Regular monitoring and documentation of carbapenems resistance is therefore crucial in developing strategies to control infections due to these bacteria in patients admitted to ICUs. Furthermore, guidelines including globally applicable infection control strategies that constitute an effective solution to reduce these infections are essential.

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