ORIGINAL ARTICLE ASIAN JOURNAL OF MEDICAL SCIENCES

Distribution of extensively drug-resistant Gram-negative bacteria, their antibiotic susceptibility profile, and the detection of metallo-β-lactamase production by them from a tertiary care center in New Delhi, India

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Submission: 19-01-2025 Revision: 02-03-2025 Publication: 01-04-2025

ABSTRACT

Background: Metallo-β-lactamase (MBL) is becoming a bigger concern worldwide because of its ability to hydrolyze carbapenems which are the drug of choice for treating severe Gram-negative bacterial infections. **Aims and Objectives:** This study aimed to estimate the percentage positivity of different gram-negative bacterial isolates, their antibiotic resistance rate, the percentage positivity of MBL production by these isolates by phenotypic method and the samples contributing to them, and the demographic profile of the patients. **Materials and Methods:** It was conducted in the Department of Microbiology, Lady Hardinge Medical College, New Delhi from November 2022 to February 2023. Two hundred seven Gram-negative extensively drug-resistant (XDR) isolates were taken from various samples and their antibiotic susceptibility profile was noted. XDR bacteria resistant to imipenem were subjected to MBL detection by the combined disc test. **Results:** Most of the patients from whom these isolates were isolated were in the age group of >18–≤65 years. Out of 207 patients, 103 were admitted in intensive care unit while the patients admitted in the ward or who came to the outpatient department were 104. *Acinetobacter baumannii* with 65.21% was the major bacteria contributing to the XDR strains. Tetracycline group of drugs showed the maximum susceptibility among the tested antibiotics and all the isolates were intermediately susceptible to colistin. Out of 207 isolates, 197 were positive for MBL and *A. baumannii* among bacteria, and tracheal aspirate among the samples was the major contributor. **Conclusion:** Most of the isolates were positive for MBL, suggesting Gram-negative carbapenem-resistant XDR isolate could be considered positive for MBL if a detection facility is not available at the center.

Key words: Gram-negative bacteria; Metallo-β-lactamase; Extensively drug-resistant; Imipenem-resistant; Combined disc test

Access this article online

Website: https://ajmsjournal.info/index.php/AJMS/index

DOI: 10.71152/ajms.v16i4.4417

E-ISSN: 2091-0576

P-ISSN: 2467-9100

INTRODUCTION

The emergence of several multidrug-resistant (MDR) organisms has become a major threat to humankind worldwide. Annually, around 70,000 deaths occur due to MDR bacteria worldwide, which will reach around 10 million by the year 2050.[1] *Escherichia coli* and *Klebsiella pneumoniae*, among *Enterobacteriaceae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, constitute the major chunk of MDR pathogens worldwide.[2]

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In the emergence of MDR pathogens, the production of β-lactamases plays the most important role. There are 4 classes of β-lactamases- A, B, C, and D, based on the amino acid sequence identity. Class B enzymes differ from others because of the presence of zinc ions on the active site and so termed metallo-β-lactamase (MBL). MBL is becoming a bigger concern worldwide because of its ability to hydrolyze carbapenems which are the drug of choice for treating severe Gram-negative bacterial (GNB) infections.[3] For carbapenem-resistant GNB, the prevalence ranges from 5.3% to 59%.[4] Early detection of MDR bacteria is crucial for the early initiation of appropriate antibiotics which will decrease the mortality rate due to these superbugs.[5]

Aims and objectives

The present study aims to estimate the percentage positivity of different bacterial isolates, their antibiotic resistance rate, the percentage positivity of MBL production by these isolates by phenotypic method and the samples contributing to them, and the demographic profile of the patients.

MATERIALS AND METHODS

This is a record-based study that included various samples that came for routine testing in the Department of Microbiology, Lady Hardinge Medical College, New Delhi from November 2022 to February 2023 from the patients admitted in the inpatient and outpatient department (OPD) of Lady Hardinge Medical College and Srimati Kalawati Saran Hospital, New Delhi. All the details required for the study were available in the Department of Microbiology. Extensively drug-resistant (XDR) bacterial isolates from pus, liver abscess, conjunctival swab, sputum, tracheal aspirate (TA), bronchoalveolar lavage, sterile body fluids, placental membrane, high vaginal swab, and drain fluid samples were included. Patients of all age groups were included in the study. Patients were categorized according to age, sex, and critical-non-critical group. This study got ethical clearance from the institutional ethical committee before the beginning of the study.

Samples were processed according to the standard protocol. Aspartate aminotransferase (AST) was performed by Kirby–Bauer disc diffusion method according to the CLSI guidelines, and susceptibility testing for colistin was done by agar dilution and broth microdilution.

XDR bacteria resistant to imipenem were considered for the phenotypic detection of MBL by the combined disc test method. For this, imipenem (10 μg) and imipenem-ethylenediaminetetraacetic acid (EDTA) (10/750 μg) discs were taken and placed 20 mm apart on Muller–Hinton agar and incubated overnight at 37°C. Isolates showing ≥7 mm increase in the diameter of zone of inhibition with imipenem-EDTA disc compared to imipenem disc were considered positive for MBL (Figure 1).

Statistical analysis was done by descriptive statistics using percentage and ratio methods and bar graphs were prepared in Microsoft PowerPoint.

RESULTS

A total of 207 XDR GNB isolates, resistant to carbapenem, were taken to detect MBL. Patient profiles were noted for those from whom these isolates were isolated. Age and sex-wise distribution is depicted in Table 1. Out of 207 patients, 103 were admitted to intensive care unit (ICU) while the patients admitted to the ward or who came to the OPD were 104.

Organisms contributing to 207 XDR GNB isolates were *A. baumannii* (65.21%), *K. pneumoniae* (20.77%), *E. coli* (8.7%), *Enterobacter* spp. (3.38%), *Citrobacter* spp. (0.96%), *Pseudomonas* spp. (0.49%), and *Proteus mirabilis* (0.49%). Antibiotic resistance rate was determined against these bacterial isolates for different tested antibiotics and it revealed that there was 100% resistance against

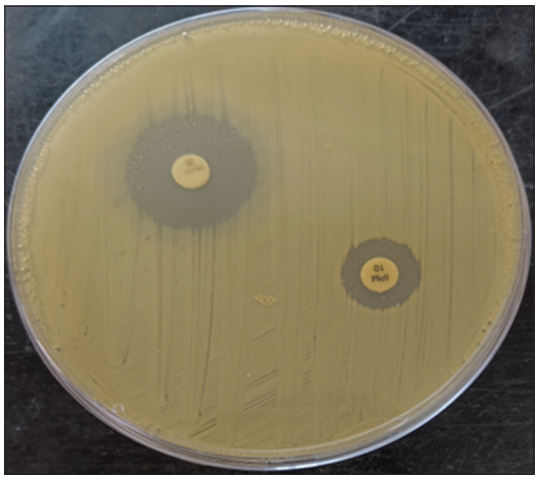

Figure 1: Combined disc test for the metallo-β-lactamase detection using imipenem and imipenem-ethylenediaminetetraacetic acid disc

Table 1: Age and sex-wise distribution of patients included in this study

	Sex	
Age	**Male**	**Female**
<2 years	15	9
≥2 to<18 years	16	18
≥18 to<65 years	56	66
≥65 years	10	17

ampicillin, ampicillin-clavulanic acid, cefixime, cefotaxime, ciprofloxacin, imipenem, and meropenem. The trend of resistance rate for other antibiotics was 99% for ceftazidime followed by 98% for piperacillin-tazobactam, 97.1% for amikacin, 95.16% for gentamicin, 83.57% for chloramphenicol, 81.64% for cotrimoxazole, 78.74% for tetracycline, 61.35% for minocycline, and 44.9% for doxycycline (Figure 2). All the isolates were intermediately susceptible to colistin.

MBL production detection by the CDT method revealed that 197/207 isolates were MBL producers.

Table 2: Sample-wise distribution of MBL production

Sample	MBL+ve (n=197)	MBL –ve (n=10)
Sputum	10	02
TA	76	01
BAL	31	02
Pus	49	01
Liver abscess	01	0
Conjunctival swab	01	0
Central line tip	03	0
Drain fluid	12	02
Sterile body fluid (CSF)	05 (02)	02 (0)
Placental membrane	02	0
HVS	07	0

CSF: Cerebrospinal fluid, MBL: Metallo-β-lactamase, TA: Tracheal aspirate, BAL: Bronchoalveolar lavage

Table 3: Organism-wise distribution of MBL production

Organism	MBL producer (n=197)	MBL non-producer (n=10)
Acinetobacter baumannii	135	0
Klebsiella pneumoniae	39	4
Escherichia coli	15	03
Enterobacter spp.	05	02
Citrobacter spp.	02	0
Pseudomonas spp.	01	0
Proteus mirabilis	0	01

MBL: Metallo-β-lactamase

Table 2 depicts the distribution of MBL-producing and non-producing bacterial isolates in different types of samples. Among the bacterial isolates, *Acinetobacter* had the maximum number of strains showing MBL production, while *P. mirabilis* did not show MBL production (Table 3).

DISCUSSION

After the advent of antibiotics, their misuse became a major concern which eventually led to an increase in antibiotic resistance worldwide. For this resistance, beta-lactamases play the major role and reports suggest that 19–60% is the prevalence range of ESBL-producing GNB and 5.3–59% is that of carbapenem-resistant GNB.[1,6]

In our study, 207 XDR Gram-negative isolates, resistant to carbapenem, were taken for MBL detection. Most of the patients from whom these isolates were isolated were in the age group of >18–≤65 years. Male: female ratio was 0.88 showing no specific gender-based difference. An almost equal number of patients fell into critical and non-critical groups which suggests that XDR bacteria are not only restricted to ICUs but also are now circulating in communities also.

A. baumannii was the major bacteria contributing to the XDR isolates followed by *Enterobacteriaceae* and *Pseudomonas* spp. Among *Enterobacteriaceae*, *K. pneumoniae* was the major pathogen followed by *E. coli*, *Enterobacter* spp., *Citrobacter* spp., and *P. mirabilis*. This is consistent with the findings of other studies where among MDR-GNB, *Acinetobacter* spp. contributed more than 60% followed by *Enterobacteriaceae*.[1] In another study done in Mumbai, the prevalence was 18.5% for drug-resistant *Enterobacteriaceae*[7] In our study, *Pseudomonas* spp. was isolated much less frequently than in other studies. This might be because blood and urine samples were not included, and only XDR isolates were taken for the study.

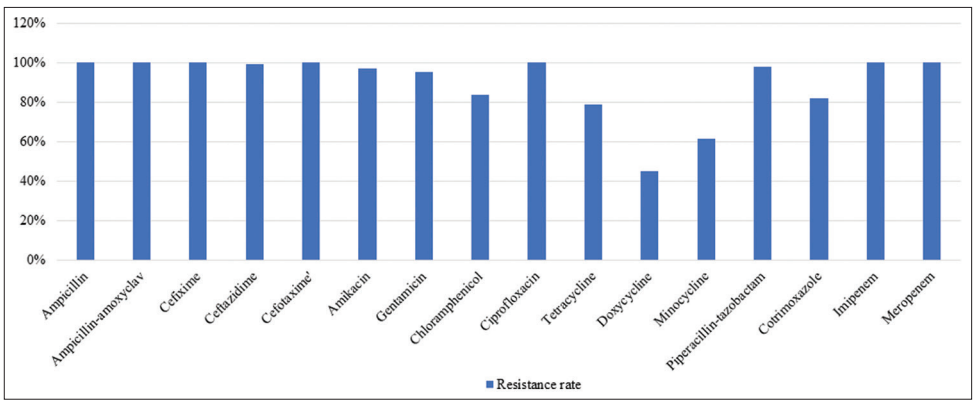

Figure 2: Antibiotic resistance rate against the tested bacterial isolates

AST of the bacterial isolates showed that susceptibility was maximum for the tetracycline group of drugs. The trend was maximum for doxycycline, followed by minocycline and tetracycline. Cotrimoxazole, chloramphenicol, amikacin, gentamicin, and piperacillin-tazobactam were other antibiotics that showed some susceptibility. No isolate was found resistant to colistin. Rest all other antibiotics did not show any susceptibility against the tested isolates. Findings of another study conducted in southern India also revealed that beta-lactam inhibitors, cephalosporins, and fluoroquinolones had the highest rates of non-susceptibility.[8] Most of the XDR bacterial strains being susceptible only to the tetracycline group of drugs poses a major challenge to treating the pediatric population as tetracyclines are not recommended in that age group.

In the present study, 95.17% of isolates produced MBL by phenotypic method. The respiratory sample, mainly TA followed by pus, was the major contributor to XDR strains and the strains producing MBL. A conjunctival swab sample also showed the production of MBL, which is not so common. Two out of 5 sterile body fluid samples were of cerebrospinal fluid, and both were positive for MBL. The isolation of MBL-producing strain from the placental membrane might pose a great threat as both the mother and child are at stake. According to another study conducted in a tertiary care hospital situated in North India, the production of MBL by *A. baumannii* from various samples was maximum by TA followed by ET secretions, pus and urine, blood, and least by sputum.[6]

In this study, 100% isolates of *A. baumannii*, *Citrobacter* spp., and *Pseudomonas* spp., 90.7% isolates of *K. pneumoniae*, 83.33% isolates of *E. coli*, 71.42% isolates of *Enterobacter* spp., and 0% isolates of *P. mirabilis* were positive for MBL. In a study done on clinical isolates of various samples, 21.8% isolates of *P. aeruginosa* and 44.8% isolates of *A. baumannii* were positive for MBL.[6] In another study conducted by a tertiary care center in Mumbai on carbapenem-resistant clinical isolates, 75.22% of isolates showed production of MBL and it was also the most prevalent carbapenemase. This study also pointed out that there was 98.9% concordance in the detection of MBL by phenotypic tests and molecular methods.[7] According to a study done in southern India, 75% of all imipenem-resistant isolates were positive for MBL.[9] In our study, this much higher rate of MBL-positive isolates might be because we took only XDR isolates.

Limitations of the study

Though the phenotypic method for detecting MBL is highly sensitive, it would have been better if it had been correlated with the molecular method.

CONCLUSION

The present study shows that an almost equal number of Gram-negative XDR strains fell into critical and non-critical groups indicating that XDR strains are now not limited to ICUs only and strict community-based surveillance is required. *A. baumannii* was the most prevalent strain. Most of the isolates were positive for MBL, suggesting Gram-negative carbapenem-resistant XDR isolate could be considered positive for MBL if a detection facility is not available at the center.

ACKNOWLEDGMENT

We would like to thank our junior residents, Dr. Anjan Das and Dr. Sandeep Kumar Yadav, and technical staff, Diwakar Mishra and Nisar, who spare their valuable time for this study.

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Source of Support: Nil, **Conflicts of Interest:** None declared.