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Original Research Article

Prevalence and Antibiotic Susceptibility of Extended-Spectrum Beta-Lactamase (ESBL) And Metallo-Beta-Lactamase (MBL) Producing Gram-Negative Bacterial Isolates in a Tertiary Care Hospital, South Delhi

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Abstract:

Extended-spectrum beta-lactamase (ESBL) and metallo-beta-lactamase (MBL) producing gram-negative bacteria (GNB) are a major cause of treatment failure in hospital and community settings, making local surveillance essential for guiding empirical therapy. This six-month cross-sectional study was conducted in the Department of Microbiology, HIMSR, and associated HAHC Hospital, Jamia Hamdard, New Delhi, between October 2023 and March 2024. A total of 280 gram-negative isolates from clinical specimens (urine, pus, blood, stool, body fluids and others) of outpatients and inpatients were identified using VITEK 2. Isolates were screened for ESBL and MBL by VITEK and disk-diffusion, and screen-positive isolates were confirmed by the Double Disk Diffusion Test (ESBL) and the Imipenem-EDTA Combined Disk Synergy Test (MBL). A subset of ESBL-positive and ESBL-negative isolates was further characterized by PCR for blaSHV, blaTEM and blaCTX-M genes. E. coli and Klebsiella spp. predominated, mostly from urine (47.8%) and pus (30.7%) samples. Of 280 isolates, 120 (42.8%) were confirmed ESBL producers and 47 (16.7%) were confirmed MBL producers, with E. coli leading both groups. Carbapenems, amikacin and nitrofurantoin retained the highest activity against ESBL producers, while colistin (95.7% sensitive) was most active against MBL producers, most of which resisted carbapenems. On PCR, blaCTX-M (56%) was the commonest ESBL gene, followed by blaTEM (44%); blaSHV was undetected. The study demonstrates a substantial burden of ESBL and MBL-producing GNB in this tertiary-care setting and underscores the need for continued surveillance, antimicrobial stewardship, and judicious carbapenem and colistin use.

Keywords: ESBL; MBL; Gram-Negative Bacteria; Antibiotic Susceptibility; blaCTX-M; blaTEM; Carbapenem Resistance.

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Introduction

Gram-negative bacteria are among the leading causes of hospital- and community-acquired infections worldwide, with Escherichia coli and Klebsiella pneumoniae listed among the World Health Organization's priority multidrug-resistant pathogens [1]. An estimated 70% of hospital-acquired infective organisms show at least partial resistance to first-line antibiotic agents [2].

Beta-lactam antibiotics — penicillins, cephalosporins, monobactams and carbapenems — act by binding penicillin-binding proteins (PBPs) in the bacterial cell wall[3]. Resistance to this class is driven largely by beta-lactamase enzymes. Extended-spectrum beta-lactamases (ESBLs), first described in the early 1980s, are plasmid-mediated enzymes (commonly encoded by blaSHV, blaTEM and blaCTX-M) that hydrolyse extended-spectrum cephalosporins, penicillins and aztreonam [4,5]. Metallo-beta-lactamases (MBLs) are a distinct, metal-ion-dependent group capable of hydrolysing carbapenems, and their spread is associated with high morbidity, mortality and healthcare cost [6,7].

Both phenotypic (disk-diffusion-based screening and synergy/confirmatory tests) and genotypic (PCR-based) methods are used to detect ESBL- and MBL-producing organisms, with molecular testing regarded as the gold standard [8], and ESBL-producing Enterobacteriaceae are increasingly recognized as an emerging public-health concern in both hospital and community settings [9]. Against this background, the present study was undertaken to determine the prevalence of ESBL- and MBL-producing gram-negative isolates from clinical samples, to evaluate phenotypic and genotypic

detection methods, and to characterize antibiotic susceptibility patterns to inform treatment planning.

Materials and Methods

Study design and setting: This cross-sectional study was carried out in the Department of Microbiology, HIMSR, and its associated HAHC Hospital, Jamia Hamdard, New Delhi, over a period of six months (October 2023 to March 2024). All clinical samples (blood, urine, pus, body fluids, etc.) received at the hospital during the study period were screened for eligibility, and a sample size of 280 isolates was estimated. Paediatric and adult patients attending various clinical departments of the hospital were included; duplicate samples were excluded. Ethical approval was obtained from the Ethics and Research Committee, Jamia Hamdard University.

Identification and antimicrobial susceptibility testing: Gram-negative isolates were identified and subjected to antimicrobial susceptibility testing using the VITEK 2 automated system (bioMérieux).

Phenotypic detection of ESBL: Screening: Each isolate was screened by VITEK 2 and the standard Kirby-Bauer disk-diffusion method as per CLSI guidelines[10], using a 0.5 McFarland bacterial suspension on Mueller-Hinton Agar with cefotaxime (30 µg), ceftazidime (30 µg) and ceftriaxone (30 µg) discs. Reduced zones of inhibition (cefotaxime <27 mm, ceftazidime <22 mm, ceftriaxone <25 mm) flagged a potential ESBL producer.

Confirmation: Screen-positive isolates were confirmed by the Double Disk Diffusion Test, comparing zones for ceftazidime and cefotaxime alone versus in combination with clavulanic acid (10 µg). A ≥5 mm increase in zone diameter with clavulanic acid confirmed ESBL production. K. pneumoniae ATCC 700603 and E. coli ATCC 25922 served as positive and negative controls, respectively.

Genotypic detection of ESBL: Phenotypically confirmed ESBL isolates were tested by PCR for blaSHV, blaTEM and blaCTX-M genes. Bacterial DNA was extracted from overnight BHI-broth cultures by the phenol–chloroform method and quantified using a Nanodrop spectrophotometer. Singleplex PCR was performed with gene-specific primers and a commercial master mix; thermal cycling used gene-specific denaturation/annealing/extension profiles (32 cycles). Amplicons were resolved on 2% agarose gel electrophoresis (ethidium bromide staining, 50 V) alongside a 100 bp ladder and positive/negative controls, and visualized using a Gel-Doc system.

Phenotypic detection of MBL: Screening: Isolates were screened for MBL production by VITEK 2 and standard disk-diffusion; isolates resistant to imipenem or meropenem were considered MBL-screen-positive.

Confirmation: Screen-positive isolates were confirmed by the Imipenem–EDTA Combined Disk Synergy Test. Two imipenem and two meropenem discs were placed on inoculated MHA; 10 µL of 0.5 M EDTA (pH 8.0) was added to one disc of each pair, and plates were incubated at 35°C for 16–18 hours. An increase in zone diameter of ≥7 mm around the EDTA-supplemented disc compared with the plain disc was considered MBL-positive.

Results

Distribution of isolates: Of 280 gram-negative isolates, 198 (70.7%) were from inpatients (IPD) and 82 (29.3%) from outpatients (OPD). Isolates were most frequently recovered from urine (47.8%) and pus (30.7%), followed by blood (10.7%), body fluids (6.07%), stool (2.85%) and miscellaneous specimens (1.8%) such as high vaginal swab, sputum, endotracheal aspirate, biopsy and tissue.

ESBL screening and confirmation: Of 280 isolates, 167 (59.6%) screened positive for ESBL production by VITEK/disk-diffusion. Among screen-positive isolates, E. coli predominated (95/167, 56.8%), followed by Klebsiella spp. (38/167, 22.7%), P. aeruginosa (11/167, 6.58%), S. Typhi (8/167, 4.8%), Citrobacter spp. and A. baumannii (4/167 each, 2.39%), Proteus spp. (2/167, 1.12%) and M. morganii (1/167, 0.59%).

On confirmatory Double Disk Diffusion testing, 120 of 167 screen-positive isolates (71.9%) were confirmed as ESBL producers — an overall ESBL prevalence of 42.8% among all 280 isolates. E. coli again predominated among confirmed producers, followed by Klebsiella spp.

Age- and gender-wise distribution: ESBL producers were most frequent among patients aged 31–40 years and least frequent in the 81–90-year age group. Of the 120 confirmed ESBL producers, 67 (55.8%) were from female patients and 53 (44.1%) from male patients. ESBL positivity by specimen type was highest among urine isolates (46.6%), followed by pus.

MBL screening and confirmation: Of 280 isolates, 55 (19.6%) screened positive for MBL production. P. aeruginosa, Acinetobacter baumannii, K. pneumoniae and E. coli were the leading MBL screen-positive organisms.

On confirmatory Imipenem–EDTA Combined Disk Synergy testing, 47 of 55 screen-positive isolates (85.5%) were confirmed as MBL producers — an overall MBL prevalence of 16.7% among all 280

isolates. Among the 47 confirmed MBL producers, males predominated (65.9%) over females (34%). MBL positivity was highest among pus samples (55.31%), followed by urine (25.5%), blood (12.7%), miscellaneous samples (8.5%) and stool (6.38%).

Antibiotic susceptibility patterns: Across all gram-negative isolates, high resistance was seen to ampicillin, ceftazidime, ceftriaxone and fluoroquinolones (ciprofloxacin, norfloxacin), while carbapenems (meropenem, imipenem, ertapenem), amikacin and nitrofurantoin retained comparatively higher sensitivity.

Table 1: Antibiotic susceptibility of ESBL vs. non-ESBL producing isolates.

Antibiotic	ESBL producers – sensitive (%)	Non-ESBL – sensitive (%)
Ampicillin	14.1%	85.9%
Amoxicillin/Clavulanic acid	45.65%	54.3%
Piperacillin/Tazobactam	51.8%	48.1%
Cefoxitin	16.5%	83.5%
Cefoperazone/Sulbactam	28.8%	71.2%
Amikacin	31.6%	89.3%
Gentamicin	34.2%	29.4%
Ciprofloxacin	54.3%	45.6%
Norfloxacin	44.1%	73.3%
Imipenem	75.8%	88.75%
Nitrofurantoin	85.7%	89.74%
Trimethoprim/Sulfamethoxazole	44.2%	55.8%

The highest sensitivity among ESBL producers was seen for nitrofurantoin (85.7%) followed by imipenem (75.8%); among non-ESBL producers, amikacin (89.3%), cefoxitin (83.5%) and ceftriaxone (83.12%) were most active. Carbapenems and aminoglycosides were overall the most reliable agents against ESBL-producing isolates.

Among MBL producers, high resistance was observed to meropenem (83%), imipenem (89.3%), amoxicillin/clavulanic acid (59.6%) and ceftriaxone (57.44%); trimethoprim-sulfamethoxazole retained only 42.5% activity. Colistin was the most active agent against MBL producers (95.7% sensitive).

Genotypic (PCR) findings: PCR was performed on 25 randomly selected phenotypically confirmed ESBL-positive isolates and 25 randomly selected ESBL-negative isolates for blaSHV, blaTEM and blaCTX-M. Among ESBL-positive isolates, blaCTX-M was detected in 14/25 (56%) and blaTEM in 11/25 (44%); blaSHV was not detected in any ESBL-positive isolate. Among the 25 ESBL-negative isolates, blaTEM was detected in 3 (12%) and blaCTX-M in 1 (4%), indicating that 4/25 (16%) phenotypically ESBL-negative isolates carried one or more ESBL genes.

Discussion

The rise of antibiotic resistance among gram-negative bacteria (GNB) reflects the ability of these organisms to acquire, maintain and express genetic material conferring resistance, at a pace that has outstripped the development of new antibiotics. Widespread empirical use of beta-lactams has selected for ESBL- and MBL-producing strains in both community and hospital settings, making ongoing surveillance and infection-control measures essential.

In this study, ESBL prevalence among gram-negative isolates was 42.8%, within the wide range reported across India by other tertiary-care studies [12,13,14,15]. This closely corroborates the 40.3% prevalence reported from South India [16], while being higher than the 30–35% range typically reported from Nepal [17] and lower than the 51.6% reported from the Gaza strip18. ESBL positivity was notably higher among urinary isolates, consistent with the well-recognized role of the urinary tract as a reservoir for ESBL-producing pathogens [11], and E. coli and Klebsiella spp. were confirmed as the predominant producers, in line with earlier work [19,20].

MBL prevalence in this study was 16.7%, again falling within the broad range reported nationally. This closely mirrors the 17.4% reported in another Indian cohort [21], while being lower than the 40% and 19.6% reported elsewhere [22,23]. P. aeruginosa, Acinetobacter spp., K. pneumoniae and E. coli were the leading MBL producers, consistent with an earlier ICU-based series [24], and pus and urine specimens carried the highest MBL burden.

Demographic analysis showed ESBL producers concentrated in the 31–40-year age group, tapering in the elderly, potentially reflecting differences in healthcare exposure, antimicrobial use, and immune status across age groups; ESBL carriage was marginally more frequent among female patients, while MBL-producing isolates were more

frequent among male patients — a disparity that warrants further study.

Antibiotic susceptibility testing confirmed high resistance to ampicillin, ceftazidime, ceftriaxone and fluoroquinolones among ESBL producers, while carbapenems, amikacin and nitrofurantoin remained comparatively effective — consistent with earlier reports that identified amikacin and imipenem as drugs of choice for ESBL-positive strains [25,26]. Among MBL producers, resistance extended to carbapenems and aminoglycosides, with colistin remaining the most reliable agent, in agreement with other reports describing preserved colistin activity despite extensive beta-lactam and aminoglycoside resistance.

The combination of screening (VITEK/disk-diffusion) followed by confirmatory phenotypic testing (Double Disk Diffusion for ESBL; Imipenem–EDTA Combined Disk Synergy for MBL) aligns with CLSI guidelines [10], and the high confirmation rate among screen-positive isolates (71.9% for ESBL) supports the reliability of this approach. Prior comparative work has similarly found the Imipenem-EDTA combined disk method to have the highest sensitivity for MBL detection among available phenotypic tests [27,28], and combining phenotypic with molecular confirmation improves overall detection accuracy [29].

On genotypic analysis, blaCTX-M was the predominant ESBL gene (56%), followed by blaTEM (44%), with blaSHV undetected — consistent with an earlier report from Sudan that similarly found CTX-M predominance over TEM in E. coli and Klebsiella isolates [30]. Notably, 16% of phenotypically ESBL-negative isolates carried one or more ESBL genes, suggesting that co-existing chromosomal or plasmid-mediated AmpC beta-lactamase production may mask phenotypic ESBL expression and produce false-negative results — a limitation also described previously, underscoring the value of combining phenotypic and molecular testing.

Conclusion

Of 280 gram-negative isolates studied, 42.8% were confirmed ESBL producers and 16.7% were confirmed MBL producers, with E. coli the predominant organism in both groups. Carbapenems, amikacin and nitrofurantoin were the most effective agents against ESBL producers, while colistin retained the highest activity against MBL producers. blaCTX-M was the most prevalent ESBL gene on genotypic testing. These findings highlight a significant burden of ESBL- and MBL-producing gram-negative bacteria in both community- and healthcare-associated infections in this setting, and underscore the importance of continued phenotypic and genotypic surveillance, antimicrobial stewardship, and judicious use of carbapenems and colistin to limit further spread of resistance.

Conflict of Interest: None declared.

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