Research Paper

Multidrug-Resistant Gram-Negative Bacilli from Community-Acquired Infections in a Tertiary Care Hospital

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ABSTRACT

Objectives: This study aimed to isolate and identify gram-negative bacilli (GNB) from community-acquired infections (CAIs) and determine the prevalence of multidrug-resistant (MDR-GNB) strains. Additionally, the study sought to characterize specific resistance phenotypes to elucidate the mechanisms driving antimicrobial resistance in community settings.

Methods: A cross-sectional study was conducted on 103 MDR-GNB isolated from 811 clinical samples between August 2022 and August 2024. Isolates were identified using morphological and biochemical tests. Antimicrobial susceptibility was determined via the Kirby-Bauer disc diffusion method, along with ESBL, MBL, and AmpC phenotype confirmation using the double-disc synergy and combined-disc assays according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Result: The results showed that 103 (33%) isolates were identified as MDR-GNB, with *Pseudomonas aeruginosa* (32%), *Escherichia coli* (28%), and *Klebsiella pneumoniae* (24%) being the most prevalent. High enzymatic resistance was observed, including AmpC-β-lactamase (53%), ESBL (51%), and MBL (42%) production, with 14% of isolates co-producing all the enzymes. Tigecycline (77%) and Gentamicin (63%) remained the most effective therapeutic agents across all resistant strains.

Conclusion: This finding highlights the escalating prevalence of MDR-GNB in the community setting. There is an urgent need for enhanced regional antibiotic management policies and continuous surveillance to combat rising resistance and preserve existing therapeutic options.

Keywords: Antimicrobial susceptibility, ABL, Community-acquired infections, ESBL, Gram-negative bacilli, MBL, Multidrug-resistant, *Pseudomonas aeruginosa*.

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Introduction

Infections caused by gram-negative bacilli (GNB) represent an escalating public health threat, particularly due to shifting antimicrobial susceptibility patterns observed over the last decade.[1,2] These organisms, including members of the families *Enterobacterales* and *Pseudomonadaceae*, are characterized by a distinct cell envelope composed of a thin peptidoglycan layer situated between the cytoplasmic and outer membranes. Among these, medically significant species such as *Escherichia coli*, *Klebsiella spp*., and *Serratia spp.* are frequently associated with CAIs. The increasing emergence of these pathogens in community settings significantly complicates clinical management and intensifies the overall burden on the healthcare system.[2]

Community-acquired infections are defined as infections contracted outside healthcare facilities or present at the time of admission. [3] These infections carry significant morbidity and mortality, necessitating early diagnosis and intervention. Between 2000 and 2015, global antibiotic consumption in low and middle-income countries rose by 39%, with India experiencing a 63% increase.[4] This surge has accelerated the prevalence of MDR organisms, particularly among GNB.

Material and Methods

This cross-sectional laboratory-based study was conducted between August 2022 and August 2024. The study included non-repetitive GNB recovered from various clinical samples obtained from outpatients during the specified period. Exclusion criteria comprised all Gram-positive organisms and any GNB isolates that did not meet the established criteria for MDR.

Collection of Data

Clinical data were prospectively collected from outpatients presenting with suspected infections at the tertiary care hospital. For each participant meeting the inclusion criteria, a structured proforma was utilized to document demographic profiles, outpatient registration numbers and primary clinical complaints. Comprehensive medical records were maintained, including relevant clinical diagnose past and present medical histories, and details of any prior or current antimicrobial therapy to ensure a thorough epidemiological assessment.

Sample size:

The sample size (n) was evaluated using a standardized formula for prevalence-based cohorts to ensure sufficient statistical power for characterizing MDR-GNB, which is □ = $4pq/L^2$. In this study, p represents the anticipated prevalence of MDR-GNB (30.77%), q is the complement (100-*p*=69.23%), and L represents the allowable error set at 10%. Based on all these parameters, the required sample size was determined to be 85.[5] To account for potential data attrition and enhance the robustness of the results, a total of 103 isolates were ultimately included in the final analysis.

Identification of Isolates

Gram-negative bacilli were identified by examining thin, heat-fixed smears stained with the Gram technique under an oil-immersion objective to characterize their morphology, size, and arrangement. Biochemical characterization was performed according to standard protocols described by Mackie and McCartney (14^{th} edition).[6]

Antimicrobial Susceptibility Test:

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton (MH) agar, following 2023 CLSI guidelines. Bacterial suspensions were standardized to a 0.5 McFarland turbidity and inoculated to produce a lawn culture. A panel of 20 antibiotics was applied to the inoculated plates. Following incubation at 37°C for 16-22 hours, zones of inhibition were measured and interpreted as susceptible, intermediate, or resistant based on CLSI criteria.[7]

Phenotypic Tests

A phenotypic test for MDR-GNB was conducted using bacterial suspensions adjusted to a 0.5 McFarland turbidity standard to ensure a uniform inoculum of approximately 1.5×10^8 CFU/ml. Isolates were initially screened for extended-spectrum beta-lactamase (ESBL) production by evaluating reduced susceptibility to indicator cephalosporins (cefotaxime, ceftazidime, ceftriaxone, and cefpodoxime) and aztreonam according to CLSI M100 criteria. Presumptive ESBL producers (e.g., ≤ 27mm for aztreonam 30µg) were confirmed using the phenotypic combined disc test; a ≥ 5mm increase in the zone of inhibition for the ceftazidime-clavulanic acid (30/10 µg) disc compared to ceftazidime (30µg) alone confirmed ESBL production.[8] Metallo-beta-lactamase (MBL) production was confirmed using the imipenem-EDTA combined disc diffusion test, where a ≥ 7mm zone increase for the imipenem-EDTA (10/750 µg) disc over imipenem (10µg) alone was considered positive.[9] Quality control was maintained using *K. pneumoniae* ATCC BAA-2146 (positive) and *E. coli* ATCC 25922 (negative). Finally, AmpC-□-lactamase production was detected via the cefoxitin-cloxacillin combined disc method a ≥ 4mm increase in the zone diameter for the cloxacillin-supplemented disc (30/200µg) compared to cefoxitin (30µg) alone indicated positive AmpC production.[10] All tests were conducted following aerobic incubation at 37°C for 18-24 hours.

Ethical Considerations

The research protocol was reviewed and approved by the Institutional Ethics Committee (KIMSDU/IEC/02/2023, Protocol no. 428-2022-23, Dated 15.03.2023). All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Results

811 specimens were processed, 487 (60.0%) yielded growth, comprising 178 gram-positive and 309 gram-negative isolated notably, 103 of the GNB were identified as MDR. community-acquired infections peaked in the 51-60 (n=21,22%) and 61-70 (n=20,21%) age groups. Prevalence declined in the elderly, reaching a minimum of 3% (n=3) in the 81-90 group, while the lowest overall prevalence (2%, n=2) was observed among teenagers (11-20 years). CAIs were more prevalent in males (n=63, 61%) than in females (n=40, 39%).

The distribution of CAIs by organism revealed that the most common pathogen in the population was

Pseudomonas aeruginosa, followed by *Escherichia coli* (n=29, 28%) and *Klebsiella pneumoniae* (n=25, 24%). Less frequent isolates included *Proteus mirabilis* and *Serratia marcescens* (each n=6, 6%), while *Klebsiella oxytoca* (n=2,2%), *Acinetobacter baumannii* (n=1, 1%), and *Citrobacter koseri* (n=1, 1%) were rarely detected.

Pus was the primary clinical sample (65%), followed by urine (29%), with sputum (5%) and high vaginal swabs (1%) accounting for the remaining isolates**.** Outpatient department (OPD) sample distribution revealed that the majority of specimens were collected from surgery (n=59, 57%), followed by orthopedics (n=24, 23%). The remaining samples were distributed among medicine (n=8, 8%), obstetrics and gynecology (n=7, 7%), and dermatology (n=5,5%). Table 1 shows Biochemical Characterization and Systematic Classification of Bacilli.

Table 1: Biochemical Characterization and Systematic Classification of Bacilli

Organism	Catalase	Oxidase	Indole	Methyl Red	Citrate	Urease	Triple Sugar Iron
Acinetobacter baumannii	+	-	-	-	+	v	K/K, No gas, No
Citrobacter koseri	+	-	+	+	-	v	K/A, Gas in but, H2Sin but
Escherichia coli	+	-	+	+	-	-	A/A, Gas in but, No H2S
Klebsiella pneumoniae	+	-	-	-	+	+	A/A, Gas in but, No H_2S
Klebsiella oxytoca	+	-	-	+	+	+	A/A, Gas in butt, No H_2S
Proteus mirabilis	+	-	-	+	+	+	A/A, Gas in butt, H_2S in butt
Pseudomonas aeruginosa	+	+	-	-	+	v	K/K, No gas, No H_2S
Serratia marcescens	+	-	-	-	+	-	K/A, No gas, No H_2S

**A/A – Acid/Acid, K/K – Alkaline/Alkaline*

Antimicrobial Susceptibility Testing of ESBL, MBL, and AmpC β-lactamase Producers

Across all enzymatic phenotypes (ESBL, MBL, and AmpC), tigecycline (77%) and gentamicin (63%) emerged as the most effective therapeutic agents, with susceptibility rates ranging from 62% to 85%. Moderate sensitivity was maintained for cefoperazone (42-49%) and netilmicin (up to 40%).

In contrast, a profound resistance profile (> 80%) was observed across multiple classes, particularly affecting ampicillin, cefuroxime, and fluoroquinolones (ciprofloxacin and nalidixic acid). Notably, carbapenem resistance was the lowest among the primary treatment classes at only 43%. This resistance was highest among ESBL (70%) and MBL (81%) producers, though it remained comparatively lower in AmpC-producing strains (53%). Substantial resistance (>60%) was also documented for amoxicillin-clavulanate, piperacillin, and ceftriaxone, underscoring the limited utility of standard *β*-lactam/inhibitor combinations in these community-acquired MDR-GNB isolates (Table 2 and 3).

Table 2: Antimicrobial Susceptibility Testing

Sr. No.	Antibiotics	Susceptible (S) [%]	Resistance (R) [%]
1.	Ampicillin (10*μ*g)	4 (11)	31 (89)
2.	Piperacillin (100*μ*g)	48 (47)	55 (53)
3.	Amoxiclav (30*μ*g)	27 (43)	36 (36)
4.	Cefuroxime (30*μ*g)	17(17)	86 (83)
5.	Ceftazidime (30*μ*g)	29 (28)	74 (72)
6.	Cefotaxime (30*μ*g)	25 (31)	55 (69)
7.	Ceftriaxone (30*μ*g)	29 (37)	49 (63)
8.	Cefoperazone (75*μ*g)	56 (54)	47 (46)
9.	Imipenem (10*μ*g)	44 (43)	59 (57)
10.	Meropenem(10*μ*g)	44 (43)	59 (57)
11.	Gentamicin (10*μ*g)	65 (63)	38 (37)
12.	Netillin (30*μ*g)	47 (46)	56 (54)
13.	Tigecycline (15*μ*g)	57 (77)	17 (23)
14.	Ciprofloxacin (5*μ*g)	24 (23)	79 (77)
15.	Nalidixic acid (30*μ*g)	25 (24)	78 (76)
16.	Co-trimoxazole (25*μ*g)	32 (46)	38 (54)
17.	Nitrofurantoin (300*μ*g)	26 (87)	4 (13)

***** *g: micro-gram*

Table 3: Prevalence of Resistance Enzymes by Species

Pathogen	ESBL (%)	MBL (%)	AmpC (%)	Triple Co-production (%)
Pseudomonas aeruginosa	12	18	22	6
Escherichia coli	19	7	13	2
Klebsiella pneumoniae	15	10	12	4
Proteus mirabilis	3	3	4	2

**ESBL: Extended-Spectrum Beta-Lactamase, MBL: Metallo-beta-lactamase, ABL: AmpC-□-lactamase*

Distribution of ESBL, MBL, and AmpC *β*-lactamase Producing Gram Negative Bacilli

The distribution of resistance enzymes among the 103 MDR gram-negative isolates revealed distinct pathogen-specific patterns. *E. coli* was the primary producer of ESBLs (19%), while *P. aeruginosa* predominated in both MBL (18%) and AmpC (22%) production. Notably, *P. aeruginosa* also exhibited the highest rates of complex resistance, leading to ESBL/MBL co-production (9%), MBL/AmpC co-production (11%), and triple enzyme expression (6%).

DISCUSSION

Our study identified a distinct age-related distribution in CAIs prevalence, with a peak incidence in the 51-60 age group (21%). This aligns with the physiological transition toward increased comorbidities and immune senescence, which often predispose middle-aged and elderly populations to opportunistic infections. While these findings mirror trends where isolates were concentrated in the 41-60 range, they diverge from literature citing peaks in much older [3] or younger cohort.[3,9,10] Such variations likely reflect regional differences in demographic vulnerability and environmental exposure. Furthermore, the observed male predominance (61%) is consistent with previous reports documenting similar ratios.[9,11]

In terms of microbial profiles, *P. aeruginosa* (32%) emerged as the most prevalent non-fermenting isolate, while *E. coli* (n=29,29%) and *K. pneumoniae* (n=25,24%) were the predominant among the *Enterobacterales.* Although our *E. coli* prevalence aligns with recent studies citing ranges of 31.4% to 39.5%. [9,12] Notably, the majority of isolates were recovered from pus (65%) and urine (29%), with a high contribution from the surgery (57%) and orthopedics (23%) departments. This suggest community-acquired skin and soft tissue infections are a primary driver of MDR-GNB prevalence in this specific clinical setting.[10]

The escalating challenge of antimicrobial resistance was particularly evident in our susceptibility data. While tigecycline (77%) and gentamicin (63%) demonstrated the highest overall susceptibility. Our observed carbapenem susceptibility (43%) was markedly lower than the 80% to 100% documented in several studies [10,13], indicating a local escalation in resistance. This is further evidenced by our ceftazidime resistance (72%), which was nearly double that of the cited literature (37.53%).[14] The high resistance to cefuroxime (83%) and fluoroquinolones (76% to 77%) underscores a significant loss of utility for these traditionally first-line empirical agents.

This resistance landscape in our cohort is driven by a complex phenotypic expression of beta- lactamases. Specifically, the phenotypic detection of ESBL (51%), MBL (42%), and AmpC (53%) production highlights a multifaceted challenge to current therapy. *P. aeruginosa* was the predominant producer of both MBL (18%) and AmpC (22%), while *E. coli* remained the primary ESBL producer. Our AmpC prevalence (53%) aligns closely with recent regional reports of 57.14% in comparable clinical environments.[15] Collectively, these findings emphasize that community-acquired MDR-GNB are increasingly harboring sophisticated enzymatic mechanisms, necessitating an urgent shift in regional empirical treatment strategies and more robust antimicrobial stewardship.

Conclusion

The presented study underscores the escalating prevalence of MDR-GNB in community-acquired infections. *P. aeruginosa* was the most frequent pathogen, with the majority of isolates recovered from surgery-related pus specimens; a substantial proportion of these were resistant strains. These findings emphasize the urgent need for continuous regional surveillance and targeted antimicrobial stewardship. Early phenotypic detection of resistance mechanisms and rational antibiotic prescribing are essential to mitigate the spread of MDR-GNB and preserve the utility of currently available therapeutic options.

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