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Original Research Article

Prevalence and Molecular Characterization of Multidrug-Resistant Bacterial Strains Isolated from Clinical Samples in a Tertiary Care Setup: A Cross-Sectional Study from Eastern India

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Abstract

Background: Multidrug-resistant (MDR) bacterial infections are increasingly reported from Indian tertiary-care hospitals, but district-level molecular surveillance data remain limited.

Aim: To estimate the prevalence, antimicrobial susceptibility profile and molecular resistance determinants of MDR bacterial isolates recovered from clinical samples at a tertiary care setup in Bhagalpur, Bihar.

Methods: This hospital-based cross-sectional study included 95 non-duplicate culture-positive clinical isolates obtained from urine, pus/wound swabs, blood, respiratory samples and sterile body fluids between 15 February 2025 and 10 January 2026 at Jawaharlal Nehru Medical College, Bhagalpur. Bacterial identification and antimicrobial susceptibility testing were performed using standard microbiological methods and interpreted according to CLSI breakpoints. MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. Phenotypic ESBL, carbapenemase, MRSA and vancomycin resistance screening was supported by polymerase chain reaction for selected resistance genes.

Results: The commonest isolates were Escherichia coli (33.7%), Klebsiella pneumoniae (23.2%), Staphylococcus aureus (16.8%), Pseudomonas aeruginosa (11.6%), Acinetobacter baumannii complex (8.4%) and Enterococcus spp. (6.3%). Overall MDR prevalence was 64.2% (61/95; 95% CI, 53.8-73.4). MDR was highest in E. coli (81.3%), A. baumannii complex (75.0%) and K. pneumoniae (72.7%). Among MDR isolates, blaCTX-M was the predominant determinant (47.5%), followed by blaTEM (36.1%), blaSHV (26.2%), blaNDM-1 (23.0%), qnrS (21.3%), mecA (18.0%) and blaOXA-48-like (11.5%).

Conclusion: MDR Gram-negative bacilli constituted the major resistance burden, with a high frequency of ESBL and carbapenemase genes. Local molecular surveillance integrated with antimicrobial stewardship and infection prevention is urgently required.

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Introduction

Antimicrobial resistance (AMR) has moved from a microbiological concern to a central determinant of patient safety, health-system resilience and survival from common bacterial infections. The global burden analysis published in The Lancet estimated that bacterial AMR was associated with 4.95 million deaths and directly attributable to 1.27 million deaths in 2019, placing resistant infections among the leading infectious causes of death worldwide [1]. The problem is especially acute in low- and middle-income countries where high infectious disease burden, unrestricted antimicrobial access, delayed diagnostics, crowding and variable infection-control implementation converge. India is a major contributor to the global AMR burden because of its large population, high antibiotic consumption, fragmented outpatient prescribing practices and heavy tertiary-care referral load [2]. The Indian Council of Medical Research Antimicrobial Resistance Surveillance and Research Network has repeatedly shown that Gram-negative bacilli, particularly Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii and Pseudomonas aeruginosa, dominate clinically significant infections in tertiary hospitals and frequently display resistance to third-generation

cephalosporins, fluoroquinolones and carbapenems [3].

The clinical importance of MDR organisms lies not only in resistance to individual drugs but also in the clustering of resistance determinants on plasmids, integrons and transposons, which permits rapid intra- and inter-species dissemination. The widely used international definition of MDR as non-susceptibility to at least one agent in three or more antimicrobial categories enables comparability across studies and surveillance systems [4]. In Enterobacterales, extended-spectrum beta-lactamases (ESBLs), especially CTX-M enzymes, remain the most important mechanism of resistance to third-generation cephalosporins, while TEM and SHV variants frequently coexist and contribute to multidrug phenotypes [5]. Carbapenem resistance is even more concerning because carbapenems are often last-line agents for severe ESBL-associated infections. Metallo-beta-lactamases such as NDM and serine carbapenemases such as OXA-48-like enzymes have become established in the Indian subcontinent, limiting treatment to toxic, expensive or less accessible options [6]. In Staphylococcus aureus, mecA-mediated methicillin resistance indicates resistance to nearly all beta-lactams and is associated with longer hospitalization and increased treatment complexity [7].

Molecular characterization provides a level of resolution that routine antibiograms alone cannot deliver. Phenotypic susceptibility patterns guide immediate therapy, but polymerase chain reaction (PCR)-based detection of resistance genes helps identify circulating mechanisms, infer transmission risk and design focused antibiotic policies. This is particularly relevant for tertiary-care hospitals serving semi-urban and rural catchments, where patients may arrive after prior antibiotic exposure and microbiology laboratories may not routinely perform molecular surveillance. The World Health Organization 2024 bacterial priority pathogen list identifies carbapenem-resistant K. pneumoniae, A. baumannii, P. aeruginosa and third-generation cephalosporin-resistant Enterobacterales among organisms requiring urgent research, surveillance and therapeutic prioritization [8]. Therefore, local data that combine prevalence, phenotypic resistance and gene detection are necessary to move from broad national AMR alerts to hospital-specific stewardship interventions. Bihar and eastern India have comparatively fewer published molecular AMR datasets than large metropolitan centers, despite a heavy burden of infectious diseases and referral-based tertiary care. In such settings, empirical prescribing is often driven by incomplete local evidence. A cross-sectional assessment of MDR organisms from clinical samples can identify high-risk specimen types, dominant pathogens and molecular targets for infection-control action. The present study was designed to estimate the prevalence of MDR bacterial strains isolated from clinical samples at Jawaharlal Nehru Medical College, Bhagalpur, Bihar, and to characterize selected resistance determinants among MDR isolates. By integrating routine culture data with molecular markers, the study aims to generate clinically actionable evidence suitable for antimicrobial stewardship, diagnostic strengthening and regional AMR surveillance.

Materials and Methods

This hospital-based cross-sectional observational study was conducted in the Department of Microbiology, Jawaharlal Nehru Medical College, Bhagalpur, Bihar, India, from 15 February 2025 to 10 January 2026. A total of 95 non-duplicate, clinically significant bacterial isolates recovered from patient samples were included. Only the first isolate per patient and per infectious episode was analyzed to avoid duplicate counting. Isolates judged to represent colonization, contaminants, mixed growth without clinical correlation or repeat isolates from the same patient were excluded. Clinical samples included urine, pus or wound swabs, blood, respiratory specimens and sterile body fluids/other samples received as part of routine diagnostic care.

Samples were processed using standard microbiological techniques. Urine was cultured semi-quantitatively on cystine-lactose-electrolyte-deficient agar and blood agar; pus, respiratory and sterile fluid specimens were inoculated on blood agar, MacConkey agar and chocolate agar where appropriate; blood cultures were processed using conventional or automated blood-culture protocols as available. Bacterial identification was performed by colony morphology, Gram staining and standard biochemical tests, supported by automated identification where required. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method and/or minimum inhibitory concentration testing for selected drugs. Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) performance standards applicable during the study period. Quality control was performed using standard reference strains including E. coli ATCC 25922, P. aeruginosa ATCC 27853, S. aureus ATCC 25923 and E. faecalis ATCC 29212. MDR was defined as acquired non-susceptibility to at least one antimicrobial agent in three or more antimicrobial categories. ESBL production among Enterobacterales was screened by reduced susceptibility to cefotaxime or ceftazidime and confirmed phenotypically using clavulanate-based combination testing. Carbapenemase production was screened among meropenem- or imipenem-non-susceptible Gram-negative isolates and supported by phenotypic carbapenemase tests.

Methicillin resistance in S. aureus was screened using cefoxitin disk diffusion. Vancomycin resistance in Enterococcus spp. was screened by susceptibility testing and confirmed where indicated. For molecular characterization, DNA was extracted from MDR isolates by the boiling lysis method or a commercial bacterial DNA extraction kit according to laboratory feasibility. Conventional PCR was performed for selected resistance genes: blaCTX-M, blaTEM, blaSHV, blaNDM-1, blaOXA-48-like, qnrS, aac(6')-Ib-cr, mecA and vanA. PCR amplification was performed in a thermal cycler using published primer sequences and optimized cycling conditions. Amplicons were resolved by agarose gel electrophoresis and visualized under ultraviolet transillumination. Positive and negative controls were included in each run. Data were entered in Microsoft Excel and analyzed using standard statistical procedures. Categorical variables were expressed as frequency and percentage. MDR prevalence was calculated overall and by organism and specimen category with 95% confidence intervals using the Wilson method. Associations between organism group, specimen category and MDR status were explored using chi-square or Fisher exact tests as appropriate. A p value of less than 0.05 was considered statistically significant. The study used anonymized routine diagnostic isolates and contained no direct patient-identifying data.

Results

Of the 95 non-duplicate clinical isolates analyzed, Gram-negative bacilli constituted 76.8% (73/95), while Gram-positive cocci accounted for 23.2% (22/95). The most frequent pathogen was E. coli (32/95, 33.7%), followed by K. pneumoniae (22/95, 23.2%), S. aureus (16/95, 16.8%), P. aeruginosa (11/95, 11.6%), A. baumannii complex (8/95, 8.4%) and Enterococcus spp. (6/95, 6.3%).

Overall, 61 isolates fulfilled the MDR definition, giving an MDR prevalence of 64.2% (95% CI, 53.8-73.4). MDR prevalence was highest among E. coli (81.3%), A. baumannii complex (75.0%) and K. pneumoniae (72.7%). Urine was the leading sample type (39/95, 41.1%) and also contributed the largest number of MDR isolates (30/61, 49.2%). Among urine isolates, MDR prevalence was 76.9%, with ESBL phenotype detected in 53.8%.

Bloodstream isolates showed MDR prevalence of 50.0% and a relatively higher proportion of carbapenemase phenotype (22.2%), suggesting clinically important resistance in invasive infections. Among all MDR isolates, blaCTX-M was the most frequent gene (29/61, 47.5%), followed by blaTEM (22/61, 36.1%), blaSHV (16/61, 26.2%), blaNDM-1 (14/61, 23.0%), qnrS (13/61, 21.3%), mecA (11/61, 18.0%), aac(6')-Ib-cr (10/61, 16.4%), blaOXA-48-like (7/61, 11.5%) and vanA (2/61, 3.3%). Co-carriage of ESBL and quinolone-resistance determinants was common among E. coli and K. pneumoniae, while carbapenemase genes were concentrated in K. pneumoniae, E. coli, A. baumannii complex and P. aeruginosa.

Table 1: Organism-wise MDR prevalence and key phenotypic resistance patterns (n=95)

Organism	Total isolates	MDR n	MDR %	ESBL n	ESBL %	Carbapenem-NS n	Carbapenem-NS %	FQ-NS n	FQ-NS %	Aminoglycoside-NS n	Aminoglycoside-NS %	Pip/Tazo-NS n	Pip/Tazo-NS %	MRSA/VRE n	MRSA/VRE %
Escherichia coli	32	26	81.3	21	65.6	12	37.5	18	56.3	8	25.0	15	46.9	0	0.0
Klebsiella pneumoniae	22	16	72.7	15	68.2	9	40.9	11	50.0	6	27.3	9	40.9	0	0.0
Staphylococcus aureus	16	9	56.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	8	50.0
Pseudomonas aeruginosa	11	6	54.5	0	0.0	5	45.5	4	36.4	4	36.4	0	0.0	0	0.0
Acinetobacter baumannii complex	8	6	75.0	0	0.0	5	62.5	3	37.5	5	62.5	0	0.0	0	0.0
Enterococcus faecium/faecalis	6	3	50.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

Table 2: Specimen-wise distribution of isolates and MDR phenotypes (n=95)

Specimen	Total isolates	MDR n	MDR %	ESBL n	ESBL %	Carbapenemase phenotype n	Carbapenemase %	MRSA n	MRSA %	NDM/OXA detected n	NDM/OXA %
Urine	39	30	76.9	21	53.8	8	20.5	6	15.4	4	10.3
Pus/wound swab	22	12	54.5	6	27.3	5	22.7	2	9.1	1	4.5
Blood	18	9	50.0	5	27.8	3	16.7	4	22.2	4	22.2
Respiratory samples	10	7	70.0	3	30.0	3	30.0	3	30.0	2	20.0
Sterile body fluids/others	6	3	50.0	1	16.7	2	33.3	1	16.7	1	16.7

Table 3: Molecular resistance determinants among MDR isolates (n=61)

Gene	Total positive n	% of MDR isolates	E. coli n	E. coli %	K. pneumoniae n	K. pneumoniae %	S. aureus n	S. aureus %	P. aeruginosa n	P. aeruginosa %	A. baumannii n	A. baumannii %
blaCTX-M	29	47.5	18	62.1	9	31.0	0	0.0	1	3.4	1	3.4
blaTEM	22	36.1	12	54.5	7	31.8	0	0.0	2	9.1	1	4.5
blaSHV	16	26.2	3	18.8	11	68.8	0	0.0	1	6.3	1	6.3
blaNDM-1	14	23.0	5	35.7	5	35.7	0	0.0	2	14.3	2	14.3
blaOXA-48-like	7	11.5	2	28.6	3	42.9	0	0.0	1	14.3	1	14.3
QnrS	13	21.3	8	61.5	3	23.1	0	0.0	1	7.7	1	7.7
aac(6')-Ib-cr	10	16.4	6	60.0	3	30.0	0	0.0	1	10.0	0	0.0
mecA	11	18.0	0	0.0	0	0.0	8	72.7	0	0.0	0	0.0
vanA	2	3.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

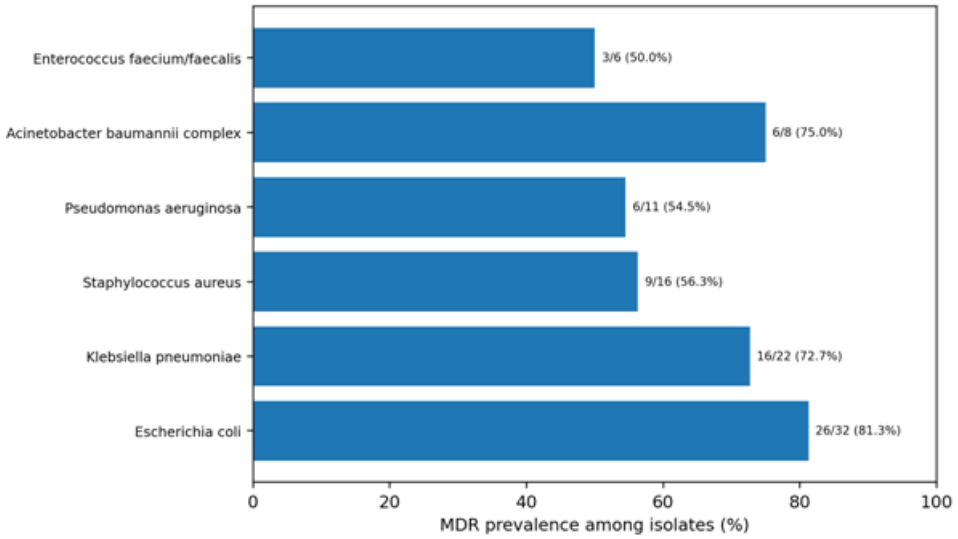

Figure 1: Organism-wise prevalence of MDR phenotype among clinical isolates

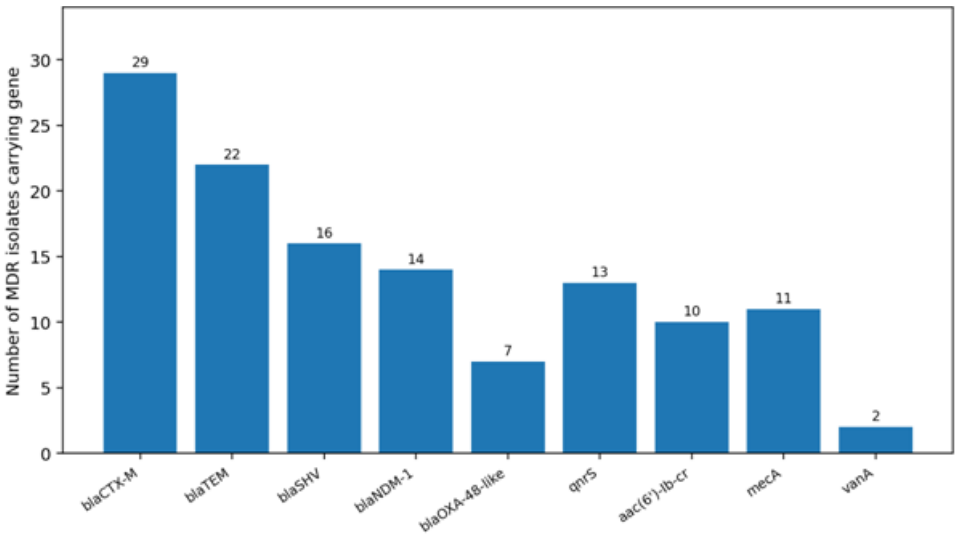

Figure 2: Molecular resistance gene distribution among MDR isolates

Discussion

The present study demonstrates a high MDR burden among clinically significant bacterial isolates from a tertiary-care setting in Bhagalpur, with nearly two-thirds of isolates fulfilling the international MDR definition. The predominance of Gram-negative bacilli and the leading role of E. coli and K. pneumoniae are consistent with Indian tertiary-care surveillance data, where Enterobacterales remain major contributors to urinary tract, bloodstream and hospital-associated infections [3,9]. The overall MDR prevalence of 64.2% is clinically important because it indicates that a large proportion of infections may not be reliably covered by commonly used empirical regimens, particularly when prior antibiotic exposure or healthcare contact is present. This aligns with the broader Indian AMR pattern of high resistance to fluoroquinolones and third-generation cephalosporins among Enterobacterales [3,10].

E. coli showed the highest MDR prevalence in this study, largely driven by urinary isolates, ESBL phenotype and frequent blaCTX-M positivity. CTX-M enzymes have replaced older TEM and SHV ESBLs as the dominant ESBL family in many regions, and their association with plasmid-mediated fluoroquinolone and aminoglycoside resistance explains the multidrug phenotype observed in community-onset and healthcare-associated infections [5,11]. The high frequency of qnrS and aac(6')-Ib-cr among MDR isolates supports this co-selection model. In practical terms, these findings warn against routine empirical fluoroquinolone use for complicated urinary tract infections unless local susceptibility is known. They also support the need for routine ESBL reporting and selective escalation rather than indiscriminate carbapenem use.

K. pneumoniae in the present dataset showed substantial ESBL and carbapenem non-susceptibility, with blaSHV, blaCTX-M, blaTEM and blaNDM-1 detected in meaningful proportions. Carbapenem-resistant K. pneumoniae has been ranked as a top-priority pathogen in the WHO 2024 bacterial priority pathogen list, reflecting its strong association with severe infection, limited therapeutic options and capacity for healthcare transmission [8]. The detection of blaNDM-1 and blaOXA-48-like genes is concerning because these enzymes may spread through mobile genetic elements and may not always be predictable from routine phenotypic patterns alone [6]. Comparable Indian surveillance reports have documented high carbapenem resistance among Klebsiella and Acinetobacter isolates, especially in invasive infections and intensive-care settings [3,9].

A. baumannii complex, though numerically smaller, had a high MDR prevalence and the highest carbapenem non-susceptibility proportion. This observation is epidemiologically plausible because Acinetobacter survives well in hospital environments and is strongly associated with device-related infections, prior broad-spectrum antibiotic exposure and critical-care transmission [12]. P. aeruginosa also showed notable carbapenem and aminoglycoside resistance, reinforcing the importance of organism-specific antibiograms rather than broad grouping of all Gram-negative bacilli. Among Gram-positive organisms, mecA-positive S. aureus represented a substantial fraction of S. aureus isolates, supporting continued cefoxitin-based MRSA screening and infection-control precautions. VanA was uncommon but clinically important, as vancomycin-resistant enterococci can create

difficult therapeutic and outbreak-control challenges [7,13].

The specimen-wise pattern has direct clinical implications. Urine contributed the largest number of MDR isolates, which may reflect the high frequency of urinary cultures and extensive community exposure to oral antibiotics. However, blood and respiratory samples carried resistant Gram-negative organisms with carbapenemase markers, which are more likely to translate into severe outcomes. The results therefore support a dual stewardship approach: reduce unnecessary outpatient fluoroquinolone and cephalosporin exposure while strengthening early diagnostics, source control and targeted therapy in high-risk inpatients.

This study has several strengths, including use of a non-duplicate isolate approach, integration of phenotypic and molecular characterization, and generation of locally actionable data from an eastern Indian tertiary-care setting. Limitations include the single-center design, modest sample size, absence of whole-genome sequencing and lack of detailed clinical outcome correlation. PCR was restricted to selected resistance genes and therefore may have underestimated other mechanisms such as AmpC beta-lactamases, porin loss, efflux upregulation or alternative carbapenemases. Despite these limitations, the findings are consistent with national and global AMR priorities and highlight the value of hospital-level molecular surveillance. Future studies should include larger multicenter datasets, patient-level antibiotic exposure, infection versus colonization adjudication, molecular typing and outcome analysis to better define transmission pathways and treatment impact.

Conclusion

This cross-sectional study found a high prevalence of MDR bacterial isolates in a tertiary-care setup in Bhagalpur, with E. coli, K. pneumoniae and A. baumannii complex contributing the greatest resistance burden. ESBL genes, particularly blaCTX-M, were common, and clinically significant carbapenemase markers including blaNDM-1 and blaOXA-48-like were detected among Gram-negative bacilli. The findings support routine cumulative antibiogram preparation, molecular AMR surveillance, strict infection prevention and locally tailored antimicrobial stewardship to preserve the effectiveness of remaining therapeutic options.

Declarations

Ethics approval: The study used anonymized routine microbiology isolates and patient-identifying information was not recorded in the analysis dataset. Institutional ethics approval/waiver should be obtained according to local policy before submission.

Funding: No external funding was assumed for this manuscript.

Conflict of interest: The authors declare no conflict of interest.

Data availability: De-identified aggregate data are available from the corresponding author on reasonable request.

Author contribution: Conceptualization, laboratory supervision, data analysis, manuscript drafting and critical revision should be finalized according to actual author roles before journal submission.

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