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Bacterial Profile, Antimicrobial Susceptibility Patterns, and Molecular Characterization of β-Lactam Resistance Among Clinical Isolates in a Tertiary Care Hospital

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ABSTRACT

Introduction: Hospital-acquired infections are a major global health concern, contributing to increased morbidity, prolonged hospital stays, and healthcare costs. The emergence of multidrug-resistant (MDR) organisms, driven by inappropriate antibiotic use and inadequate infection control, further complicates treatment. Gram-negative bacteria, particularly Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa, are major causes of healthcare-associated infections, while resistant Gram-positive organisms such as MRSA and VRE also pose significant threats.[1,2,5] Therefore, regular surveillance of bacterial pathogens and their antimicrobial susceptibility patterns is essential to guide empirical therapy, promote rational antibiotic use, and strengthen antimicrobial stewardship programmes.[6]

Objectives: The study aims to determine the bacterial profile and antimicrobial susceptibility patterns of clinical isolates from patients attending a tertiary care hospital. It also seeks to assess the prevalence of ESBL and AmpC β-lactamase production, detect carbapenemase activity using the Modified Carbapenem Inactivation Method (M-CIM), and identify the blaTEM and blaSHV resistance genes using molecular methods.

Results: A total of 250 clinical bacterial isolates were analyzed. The mean age of patients was 49.65 ± 16.78 years, with males accounting for 55.2% and inpatients for 73.2%. Most isolates were obtained from the Surgery department (51.6%), with pus (38.4%) and urine (27.6%) being the predominant specimens. Escherichia coli (26.8%) was the most frequently isolated organism, followed by Klebsiella pneumoniae (24.8%) and Pseudomonas aeruginosa (19.6%). Antimicrobial susceptibility testing revealed variable sensitivity and resistance patterns among the isolates. E. coli demonstrated the highest sensitivity to amikacin (39.9%), while P. aeruginosa showed high sensitivity to piperacillin–tazobactam (26.1%). Resistance was predominantly observed among E. coli and K. pneumoniae against several antibiotics. Molecular analysis revealed the presence of blaTEM and blaSHV genes in 54 (21.6%) and 44 (17.6%) isolates, respectively. K. pneumoniae was the predominant organism harboring both blaTEM (44.5%) and blaSHV (36.4%) genes, followed by E. coli. These findings highlight the considerable prevalence of antimicrobial resistance and β-lactamase-producing organisms among clinical isolates.

Conclusion: The predominance of E. coli and K. pneumoniae, coupled with diverse antimicrobial resistance mechanisms, highlights the urgent need for continuous surveillance, judicious antibiotic use, and robust infection-control measures to combat antimicrobial resistance.

Keywords: Bacterial Profile, Antimicrobial Susceptibility Patterns, β-Lactam Resistance.

INTRODUCTION

Hospital-acquired infections pose a significant challenge for healthcare systems globally, leading to increased patient morbidity, extended hospital stays, and higher healthcare costs. The rise of multidrug-resistant (MDR) organisms complicates infection management, often restricting treatment options and resulting in poorer outcomes. Antimicrobial resistance has emerged as a critical global public health issue, underscoring the necessity for ongoing surveillance of bacterial pathogens and their resistance trends. Research indicates that the inappropriate and excessive use of antibiotics, coupled with insufficient infection control practices, significantly contributes to the emergence and spread of resistant organisms.1,2

Recent studies in various healthcare settings indicate a growing prevalence of Gram-negative bacteria in clinical infections. Notable pathogens include Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa, often involved in urinary tract, bloodstream, respiratory, and surgical wound infections. Their ability to develop resistance mechanisms, such as extended-spectrum β-lactamase (ESBL) production and carbapenem resistance, complicates treatment. Additionally, resistant Gram-positive bacteria, including methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococcus (VRE), are significant contributors to healthcare-associated infections, leading to severe cases, extended hospital stays, and higher mortality rates due to their limited antibiotic susceptibility.2,5

Given the rising concerns, regular evaluations of local bacterial profiles and antimicrobial susceptibility are vital for optimising empirical therapy and guiding appropriate antibiotic use. This information helps clinicians choose suitable antimicrobial agents, minimises the misuse of broad-spectrum antibiotics, and supports effective antibiotic stewardship programmes.6

Objectives:

Primary Objective

To determine the bacterial profile and antimicrobial susceptibility patterns of clinical bacterial isolates obtained from patients attending a tertiary care hospital.

Secondary Objective

To assess the prevalence of extended-spectrum β-lactamase (ESBL) and AmpC β-lactamase production among the bacterial isolates. To evaluate the phenotypic detection of carbapenemase activity using the Modified Carbapenem Inactivation Method (M-CIM). To detect the presence of blaTEM and blaSHV resistance genes among the clinical bacterial isolates using molecular methods.

Methodology:

This prospective cross-sectional , laboratory-based study was conducted in the Department of Microbiology at Government Namakkal medical college and Hospital, Namakkal over three months from August 2025 to January 2026 The study involved clinical specimens collected from various hospital departments for routine aerobic bacterial culture and antimicrobial susceptibility testing.

Eligible clinical specimens collected during the designated study period were included, resulting in a final analytical sample of 250. This sample comprised urine, pus/wound swabs, blood, respiratory specimens, body fluids, and other clinically relevant samples, provided they were properly collected, labelled, transported, and accompanied by sufficient clinical information. Specimens deemed inadequate in quantity, improperly labelled, leaking, contaminated, or unsuitable for routine bacterial culture were excluded. In cases of repeated isolates from the same patient and clinical episode, only the first clinically significant isolate was used for resistance profiling to avoid duplication.

Specimens were handled following standard bacteriological protocols relevant to their type. Samples were placed on appropriate culture media and incubated under suitable aerobic conditions. Significant growth was evaluated based on clinical and laboratory criteria. Bacterial isolates were identified through colony morphology, Gram staining, and standard biochemical tests. This identification process was backed by routine quality control measures in the laboratory.

Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disk diffusion method. Antimicrobial agents were chosen based on the organism group, specimen type, and standard laboratory protocols, with inhibition-zone diameters interpreted according to Clinical and Laboratory Standards Institute breakpoints relevant to the study period. For key Gram-negative isolates, the panel included amikacin, ceftazidime, cefepime, ciprofloxacin, piperacillin-tazobactam, and meropenem. For significant Gram-positive isolates, the relevant agents included cefoxitin (when applicable), gentamicin/high-level gentamicin, ciprofloxacin, clindamycin, linezolid, vancomycin, and ampicillin.

ESBL production in E. coli and Klebsiella isolates was evaluated phenotypically through standard screening and confirmatory methods, utilising cephalosporin-clavulanate synergy, in line with Indian multicentre surveillance practices. Methicillin resistance in S. aureus was assessed via cefoxitin susceptibility testing. Vancomycin resistance in enterococci

was noted from routine susceptibility data. Multidrug resistance was defined as non-susceptibility to at least one antimicrobial agent across three or more categories, adhering to international consensus definitions.7

Frequencies and percentages were used to summarize the data. The chi-square test was used to evaluate culture positive across specimen groups; $P<0.05$ was deemed statistically significant. The analytical dataset does not include patient identification. Before the study began, the necessary permissions were acquired.

RESULTS:

Table1: Demographic details:

Demographic details		variables(n=250)	percentage	Total
Age in years (Mean ± SD)		49.65±16.78		
Patients position	IP	183	73.2	250(100%)
	OP	67	26.8	
sex	Female	112	44.8	250(100%)
	Male	138	55.2	
Department	DERM	2	.8	250(100%)
	ENT	6	2.4	
	MED	37	14.8	
	MICU	5	2.0	
	MMW	1	.4	
	OBG	15	6.0	
	ORTHO	31	12.4	
	PAED	9	3.6	
	PEAD	1	.4	
	RESP	12	4.8	
	SICU	2	.8	
	SURG	129	51.6	
Sample	Ascitic fluid	1	.4	250(100%)
	BAL	1	.4	
	Blood	14	5.6	
	Ear swab	7	2.8	
	ET	2	.8	
	Pleural fluid	1	.4	
	Pus	96	38.4	
	Sputum	24	9.6	
	Throat swab	2	.8	
	Tissue	22	8.8	
	Urine	69	27.6	
	Wound	11	4.4	
ORGANISMS	Citrobacter Freundii	3	1.2	250(100%)
	E. Coli	67	26.8	
	Enterobacter aerogenes	19	7.6	
	Klebsiella Pneumoniae	62	24.8	
	Klebsiella Oxytoca	2	.8	
	Morganella Morganii	6	2.4	
	Proteus Mirabilis	20	8.0	
	Proteus Vulgaris	22	8.8	
	Pseudomonas aeruginosa	49	19.6	

Drug sensitivity of AMP/s (Ampicillin–Sulbactam) was high among E. Coli 26(37.1%). Drug resistant of AMP/C was high among Klebsiella 45(33.5%). drug sensitivity of GEN(Gentamicin) was high among E. Coli 51(36.7%). Drug resistant of GEN was high among E. Coli 13 (27.7%). Intermediate Response of GEN was high among Klebsiella pneumoniae 7(76.7%). Drug sensitivity of AK (Amikacin) was high among E. Coli 61(39.9%). Drug resistant of AK was high among Klebsiella pneumoniae 12(32.4%). Intermediate Response of AK was high among Enterobacter Aerogenes 5(23.8%). Drug sensitivity of PIT (Piperacillin–Tazobactam) was high among Pseudomonas aeruginosa 41(26.1%). Drug resistant of PIT was high among E. Coli 19(33.3%). that drug sensitivity of CX (Cefuroxime) was high among E. Coli 55(27.6%). Drug

resistant of CX was high among Pseudomonas aeruginosa 31(15.6%). Intermediate Response of CX was high among E. Coli 5(38.5%).Drug sensitivity of CTR was high for Klebsiella pneumoniae 31(36.1%). Drug resistant of CTR (Ceftriaxone) was high for E. Coli 39(38.2%). Intermediate Response of CTR was high among E. coli 6(40%). drug sensitivity of CIP (Ciprofloxacin) was high for Pseudomonas aeruginosa 36(28.6%). Drug resistant of CIP was high for E. Coli 39(38.6%). Intermediate Response of CIP was high for E.coli and Enterobacter aerogenes 5(21.7%). Drug sensitivity of COT(Co-trimoxazole) was high for Klebsiella pneumoniae 38(31.9%). Drug resistant of COT was high for E.Coli 33(45.8%). Intermediate Response of COT was high for Klebsiella pneumoniae 4(33.3%). drug sensitivity of CZ (Cefazolin) was high for Klebsiella pneumoniae 29(40.8%). Drug resistant of CZ was high for E.coli 41(38.3%). Intermediate Response of CZ was high for E.coli 5(23.8%). drug sensitivity, resistant, Intermediate Response of LE (Levofloxacin) was high for Pseudomonas aeruginosa 25(96.2%),15(93.8%),4(100%) respectively. drug sensitivity, resistant, Intermediate Response of NIT (Nitrofurantoin) Was high for E.Coli 16(43.2%),3(42.9%),4(40%) respectively. drug sensitivity of CAZ(Ceftazidime) was high for Pseudomonas aeruginosa 26(27.4%). Drug resistant of CAZ was high for E.coli 43(32.8%). Intermediate Response of CAZ was high for E.coli and Enterobacter aerogenes 5(20.8%). drug sensitivity of TE (Tetracycline) was high for Klebsiella pneumoniae 35(34%). Drug resistant of TE was high for E.coli 35(38.9%). Intermediate Response of TE was high for Klebsiella pneumoniae 4(44.4%). drug sensitivity, resistant, Intermediate Response of TOB (Tobramycin) was high for Pseudomonas aeruginosa 31(93.9%),9(90%),5(100%) respectively. Drug sensitivity of CPM(Cefepime) was high for E.Coli 26(26%). Drug resistant of CPM was high for Klebsiella pneumoniae 38(29%). Intermediate Response of CPM was high for Pseudomonas aeruginosa 6(37.5%). Drug sensitivity of CFM (Cefixime) was high for Klebsiella pneumoniae 38(36.5%). Drug resistant of CFM was high for E. Coli 31(39.2%). Intermediate Response of CFM was high for Enterobacter aerogenes 4(23.5%). Drug sensitivity of AZM was high for Klebsiella pneumoniae 38(38.7%). Drug resistant of AZM (Azithromycin) was high for E. Coli 39(37.5%). Intermediate Response of AZM was high for Pseudomonas aeruginosa 47(97.9%).Drug sensitivity of IMP(Imipenem)was high for E.COLI 57(29.5%). Drug resistant of AZM was high for Klebsiella pneumoniae 16(37.2%). Intermediate Response of AZM was high for Klebsiella pneumoniae 6(42.9%). Drug sensitivity of MRP was high for E. Coli 61(27.9%). Drug resistant of MRP (Meropenem) was high for Klebsiella pneumoniae 11(47.8%). Intermediate Response of MRP was high for Klebsiella pneumoniae and Enterobacter aerogenes 2(25%). Drug sensitivity of CL(Colistin) was high for pseudomonas aeruginosa 2(100%). Intermediate Response of AZM was high for Klebsiella pneumoniae 11(57.9%). drug sensitivity, resistant, Intermediate Response of TOB was high for Pseudomonas aeruginosa

Figure 1: AMPC Positive Organisms:

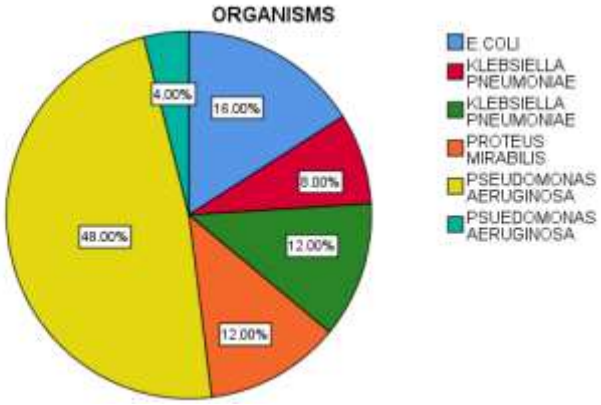


Figure 2: MCIM Positive Organisms:

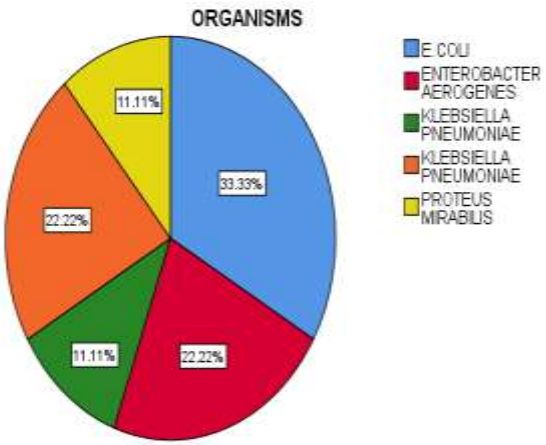


Table 2: BlaTEM Gene Detected Organisms:

Organisms	BlaTEM GENE Positive	
	Frequency	Percentage
E. COLI	14	25.9
ENTEROBACTER AEROGENES	4	7.4
KLEBSIELLA PNEUMONIAE	24	44.5
KLEBSIELLA OXYTOCA	1	1.9
PROTEUS MIRABILIS	6	11.1
PROTEUS VULGARIS	2	3.7
PSEUDOMONAS AERUGINOSA	3	5.6
Total	54	100%

Table 3: Bla SHV Gene Detected Organisms:

Organisms	Bla SHV Gene Positive	
	Frequency	Percentage
E.COLI	12	27.3
ENTEROBACTER AEROGENES	4	9.1
KLEBSIELLA PNEUMONIAE	16	36.4
KLEBSIELLA OXYTOCA	1	2.3
PROTEUS MIRABILIS	6	13.6
PROTEUS VULGARIS	2	4.5
PSEUDOMONAS AERUGINOSA	3	6.8
Total	44	100%

DISCUSSION:

Our study stated that A total of 250 clinical bacterial isolates were analyzed. The mean patient age was 49.65 ± 16.78 years, with males accounting for 55.2% and inpatients for 73.2%. Most isolates were obtained from the Surgery department (51.6%), with pus (38.4%) and urine (27.6%) being the predominant specimens. Escherichia coli was the most frequently isolated organism (26.8%), followed by Klebsiella pneumoniae (24.8%) and Pseudomonas aeruginosa (18.4%). Antimicrobial susceptibility varied across the bacterial isolates. E. coli showed the highest reported susceptibility to ampicillin (37.1%), gentamicin (36.7%), amikacin (39.9%), and cefuroxime (27.6%). K. pneumoniae demonstrated the highest reported susceptibility to ceftriaxone (36.1%), cotrimoxazole (31.9%), cefazolin (40.8%), tetracycline (34.0%), cefixime (36.5%), and azithromycin (38.7%). P. aeruginosa showed the highest reported susceptibility to piperacillin-tazobactam (26.1%), ciprofloxacin (28.6%), levofloxacin (96.2%), tobramycin (93.9%), and ceftazidime (27.4%). Resistance was frequently reported among E. coli and K. pneumoniae, although the exact percentages should be interpreted with caution pending verification of the denominators and drug-specific results. ESBL production was detected in 55 isolates, predominantly K. pneumoniae (43.6%), while AmpC production was identified in 25 isolates, most commonly P. aeruginosa (52%). M-CIM positivity was observed in nine isolates, mainly E. coli and K. pneumoniae (33.3% each). The blaTEM gene was detected in 54 isolates, with K. pneumoniae accounting for 44.5%, while blaSHV was detected in 44 isolates, predominantly in K. pneumoniae (36.4%). Overall, E. coli and K. pneumoniae were the predominant isolates, with notable phenotypic and molecular β-lactam resistance patterns

Dr. Misbah Manzoorudeen Kassim et al.8 stated that A total of 100 Enterobacteriaceae isolates from pus samples were analyzed. Wound infection was the most common presentation (58%), with females (78%) and patients aged 21–40 years (52%) predominating. Most samples were received from Obstetrics and Gynaecology (47%). Klebsiella pneumoniae (28%) and Escherichia coli (27%) were the predominant isolates. Carbapenem resistance was detected in 41% of isolates. Piperacillin–tazobactam showed the highest sensitivity (78%), whereas ceftriaxone demonstrated the highest resistance (70%). Overall, the findings indicate a considerable burden of carbapenem resistance among Enterobacteriaceae isolated from pus samples.

Singh N 9 et al stated that A total of 1,000 clinical samples were processed, of which 464 (46.4%) were culture-positive. Urine was the most frequently received specimen (43.2%), followed by pus (15.7%) and blood (14.9%). Escherichia coli was the predominant isolate (33.19%), followed by Staphylococcus aureus (20.91%), Klebsiella pneumoniae (13.58%), and Pseudomonas aeruginosa (12.28%). Among Gram-negative isolates, E. coli and Klebsiella spp. showed relatively high sensitivity to amikacin and carbapenems, whereas Acinetobacter spp. demonstrated marked resistance to most tested antibiotics. MRSA isolates exhibited high resistance to commonly used antibiotics but remained sensitive to vancomycin and linezolid. Overall, the findings highlight the diversity of clinical pathogens and the importance of ongoing antimicrobial surveillance and antibiotic stewardship.

Vijaya Swetha V10 et al ,Among 500 clinical specimens, 318 (63.6%) were culture-positive, with Gram-negative bacteria predominating (71.7%), particularly E. coli (28.9%) and Klebsiella spp. (19.2%). Amikacin, piperacillin–tazobactam, and

meropenem showed comparatively higher susceptibility, while 40.6% of isolates were multidrug-resistant, with the highest MDR rate in Acinetobacter spp. (60%). These findings emphasize the need for ongoing antimicrobial surveillance and stewardship.

AHDI KS11 et al stated that Antimicrobial susceptibility testing through the disk diffusion method revealed that the lowest resistance rates were against polymyxin B (16%), colistin (23%), and rifampin (27%); whereas the highest resistance rate was observed against ticarcillin (100%), cefixime (100%), and ceftizoxim (100%). Screening by double disk synergy test showed that 60% of the isolates were ESBL producers. PCR technique on ESBL-positive isolates determined blaSHV gene as the most prevalent (31.6%) and blaOXA-10 as the least prevalent (8.3%) among the studied resistance genes.

The present study identified E. coli (26.8%) and K. pneumoniae (24.8%) as the predominant isolates, consistent with findings by Kassim et al., Singh et al., and Vijaya Swetha et al., who also reported the predominance of these organisms. However, antimicrobial susceptibility varied across studies, likely reflecting differences in local resistance patterns and study populations. ESBL production was detected in 55 isolates, predominantly K. pneumoniae, while AmpC production was most common in P. aeruginosa. In comparison, AHDI KS et al. reported ESBL production in 60% of isolates, with blaSHV being the most prevalent resistance gene. These findings highlight the considerable burden of antimicrobial resistance and the importance of continued surveillance and antibiotic stewardship.

CONCLUSION:

The present study highlights the predominance of E. coli and K. pneumoniae among clinical bacterial isolates, alongside a considerable burden of antimicrobial resistance. The detection of ESBL, AmpC, carbapenemase activity, and β-lactam resistance genes underscores the urgent need for continuous antimicrobial surveillance, rational antibiotic use, and effective infection-control measures to combat the growing threat of antimicrobial resistance.

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