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EXPLORING ANTIBIOTIC RESISTANCE MECHANISMS AND MOLECULAR EPIDEMIOLOGY IN CLINICAL ISOLATES OF GRAM-NEGATIVE BACTERIA AT A TERTIARY CARE HOSPITAL: A CROSS-SECTIONAL STUDY

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ABSTRACT

Antimicrobial resistance (AMR) is a serious global health concern, owing mostly to the rapid spread of resistant Gram-negative bacteria in hospital settings. This study investigated the antibiotic resistance patterns, molecular resistance mechanisms, and genetic variables of Gram-negative clinical isolates obtained from a tertiary care hospital in Tamil Nadu, India. A cross-sectional study was undertaken at the Government Theni Medical College and Hospital between November 2024 and April 2025. 171 bacterial isolates were collected from 219 clinical specimens, with 142 Gram-negative isolates chosen for further investigation.

The Kirby-Bauer disk diffusion method was used to conduct phenotypic antimicrobial susceptibility testing in accordance with the Clinical and Laboratory Standards Institute guidelines. Carbapenemase-encoding genes like blaOXA-51 and blaOXA-23 were found using real-time polymerase chain reaction (RTPCR). GraphPad Prism software was used to evaluate the clinical, microbiological, and demographic data. Thirty-one percent of the 142 Gram-negative isolates exhibited treatment resistance. The most resistant strain was Klebsiella pneumoniae (47.7%), followed by Escherichia coli (18.2%) and Klebsiella oxytoca (25.0%). Meropenem resistance was observed in 38.2% of K. pneumoniae isolates, indicating a significant level of resistance to cephalosporins, fluoroquinolones, and aminoglycosides. According to molecular analysis, blaOXA-51 (67.3%) and blaOXA-23 (69.1%) genes were highly prevalent in K. pneumoniae, and they were also often found in E. coli and Pseudomonas aeruginosa. Differences in cycle threshold values indicated species-specific variations in gene expression. The results show a high prevalence of multidrug-resistant Gram-negative pathogens and highlight the necessity of ongoing molecular surveillance, improved antimicrobial stewardship, and focused infection control measures to stop the spread of AMR in healthcare settings with limited resources.

Keywords: Antimicrobial resistance, Gram-negative bacteria, Klebsiella pneumoniae, Escherichia coli, Carbapenemase.

INTRODUCTION

A growing worldwide health concern, antimicrobial resistance (AMR) compromises the effectiveness of antibiotics and makes managing infectious diseases more difficult.[1] 7.7 million deaths globally were attributed to bacterial infections, according to the Global Burden of Disease (GBD) report, highlighting the critical need for concerted efforts to combat treatment resistance in bacterial pathogens.[2] AMR makes it more difficult to treat common infections, which leads to longer illnesses, more medical costs,

and higher death rates. Because they may quickly develop and disseminate resistance mechanisms, gram-negative bacteria like Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter spp. have become especially problematic.[3] Treatment options are severely limited by MDR phenotypes, carbapenem resistance, and ESBL production.[4] According to estimates from the US Centers for Disease Control and Prevention (CDC), antibiotic-resistant illnesses kill over 35,000 people annually and impact over 2.8 million people.[5] Methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococcus (VRE), and drug-resistant Mycobacterium tuberculosis are among the high-priority resistant bacteria that the World Health Organization (WHO) has designated as serious threats to human health.[6] Notably, MRSA alone is responsible for 39.1% of AMR-related mortality in middle-income countries and 32.1% in high-income nations.[7] Furthermore, environmental reservoirs, particularly aquatic areas contaminated with pharmaceutical waste and agricultural runoff, have been linked to the proliferation and selection of resistant strains, acting as conduits for transmission between animals and humans.[8]

Gram-negative bacteria are the major cause of healthcare-associated infections (HAIs) in hospitals, contributing to increased morbidity, longer hospital stays, and higher treatment costs.[9] Despite this burden, there is still a considerable dearth in molecular epidemiology data on resistance mechanisms, especially in resource-constrained settings like rural India. Understanding the genetic basis and incidence of AMR at the local level is essential for creating tailored antimicrobial stewardship measures and directing empirical treatment.[10] Investigating the antibiotic resistance patterns and underlying molecular mechanisms in Gram-negative bacterial isolates obtained from clinical specimens at Tamil Nadu's Government Theni Medical College and Hospital is the aim of this study. In order to identify common resistance patterns and comprehend the genetic factors causing antimicrobial resistance in the area, this work integrates phenotypic resistance data with molecular analyses of resistance genes, such as blaOXA-51 and blaOXA-23 variants. Additionally, data from isolate clonal relatedness will be used to guide local policy and infection control strategies.

MATERIALS AND METHODS

Study design: This cross-sectional study was carried out in a hospital to evaluate the microbiological profile and associated factors among research participants over a predetermined study period.

Study area: The study was conducted at the Department of Microbiology, Government Theni Medical College and Hospital (GTMCH), Theni, Tamil Nadu.

Study population: This study aimed to assess the antibiotic resistance patterns of pathogenic bacterial strains obtained from clinical specimens. A total of 219 clinical samples were obtained from clinically suspected patients admitted to the Government Theni Medical College and Hospital in Theni, Tamil Nadu. From them, 171 harmful bacterial strains were successfully identified and included in the final study. The study comprised 142 isolates, each of which corresponded to a particular patient.

Inclusion Criteria

1. Gram-negative bacteria found in clinical specimens, such as blood, urine, wound swabs, and respiratory samples, from patients at Govt. Theni Medical College and Hospital.
2. Patients with Gram-negative bacterial infections, both inpatients and outpatients, regardless of age.
3. Individuals who have clinical and microbiological records demonstrating a Gram-negative bacterial illness.
4. Isolates for which the results of all available antimicrobial susceptibility tests are available.

Exclusion Criteria

1. Several isolates obtained during the same illness episode from the same patient. To prevent bias, only the first isolate discovered throughout the study period will be included.
2. Isolates without clinical data required for analysis or with partial or absent results from antimicrobial susceptibility tests.
3. Isolates determined by clinical and microbiological evaluation to be contaminants rather than actual pathogens.
4. Isolates that have been classified as non-Gram-negative or Gram-positive organisms.

Sample collection and isolation of pathogens

Clinical isolates were acquired from diagnostic specimens routinely processed at the hospital microbiology laboratory over the course of the previous six months. For this investigation, the top six most often found bacterial strains were selected. Samples were taken from both inpatients and outpatients at the Government Medical College/Hospital in Theni between November 2024 and April 2025. According to Cheesbrough (2005), the designated physicians gathered samples from different wards in sterile containers, which were then promptly examined at the bacteriology lab.[11] The location and duration of specimen preservation and storage varied; for instance, blood, urine, pus, and sputum were retained until they were examined and discussed with the designated physician. For significant specimens like postoperative samples, among other things, bodily fluids were stored for seven to ten days. Alongside the sample collection, information such as gender, age, ward, collection date, specimens, and other characteristics were noted. Ten to fifteen randomly chosen samples were included in this three-month survey every day. Additionally, isolated from the samples were Pseudomonas aeruginosa, Escherichia coli,

Enterobacter spp., Klebsiella pneumoniae, Staphylococcus spp., and Acinetobacter spp.[12] After seven days, the lack of turbidity was considered detrimental. MacConkey agar plates, blood, and nourishment were used to subculture positive samples. A sterile plastic container with a 50 mL capacity was used to collect urine samples (20–30 mL), which were then streaked onto MacConkey agar, blood agar, cysteine lactose electrolyte deficient agar, and nutritional agar plates. MacConkey agar, blood, and nutrition plates were streaked with pus/swab samples. Chocolate, blood, nutritional, and MacConkey agar plates were streaked with sputum samples (2–5 mL) that were collected in a sterile plastic container with a 50 mL capacity. With the exception of blood, all samples were processed on the same day; streaked plates were subcultured on nutritional agar plates after being incubated at 37°C for 24 to 72 hours. The Gram reaction, biochemical assays, and colony morphology were used to identify the bacteria.[13]

Antibiotic susceptibility test of bacterial isolates

The Kirby-Bauer disk-diffusion method was used to assess the antibiotic susceptibility of both Gram-positive and Gram-negative bacterial isolates.[14] To create the equivalent of 0.5 McFarland standard solution, a single colony was chosen and suspended in sterile normal saline (0.85% NaCl). 19 mL of sterile Muller-Hinton soft agar (45°C) and 1 mL of bacterial solution were combined and placed to Petri dishes. After the antibiotic disks were transferred using a disk dispenser, the plates were incubated at 37°C for a whole day. Based on the degree of the zone of inhibition, bacterial isolates were categorized as resistant, intermediate, or susceptible to antibiotics using the Clinical Laboratory Standard Institute (CLSI) guidelines.[14] Antimicrobial susceptibility testing of Enterobacter spp., Klebsiella pneumoniae, and Escherichia coli isolated from pus, swab, sputum, and blood samples was performed using ciprofloxacin (CIP), levofloxacin (LVX), gentamicin (GM), amikacin (AN), meropenem (MEM), cefuroxime (CXM), cefotaxime (CTX), ceftazidime (CAZ), ceftazidime–clavulanate (CAC), cefepime (FEP), piperacillin–tazobactam (TZP), trimethoprim–sulfamethoxazole (SXT), tetracycline (TE), and ampicillin–sulbactam (SAM). Isolates obtained from urine samples were additionally tested for susceptibility to TR, NX, and nitrofurantoin (NIT). Antibiotic susceptibility of Pseudomonas aeruginosa was evaluated using imipenem–EDTA (IE), imipenem (IPM), aztreonam (ATM), ciprofloxacin (CIP), gentamicin (GM), levofloxacin (LVX), amikacin (AN), and cefepime (FEP). Staphylococcus spp. isolates showed resistance to erythromycin (E), linezolid (LZD), ciprofloxacin (CIP), rifamycin (RIF), clindamycin (CM), tetracycline (TE), vancomycin (VA), trimethoprim–sulfamethoxazole (SXT), gentamicin (GM), chloramphenicol (C), meropenem (MEM), cefoxitin (FOX), and penicillin.

Molecular Characterization of Antibiotic Resistance

DNA extraction: Following 24 hours of bacterial growth on a culture plate, genomic DNA was extracted using a DNA extraction kit (Microbial DNA | Bacterial DNA Isolation Kits, QIAGEN) as directed by the manufacturer.

Real-time PCR assay: This study focused on the expression of various genes extracted from bacteria (for example, blaOXA-51, blaOXA-23, blaOXA-24, blaOXA-58, blaOXA-48, blaNDM-1, and blaKPC). The CFX96™ Real-Time PCR Detection System (BioRad, Hercules, California 94547 USA) was utilized for all RT-PCRs. Every RT-PCR amplification reaction was carried out in 96-well plates with a final volume of 25 µL (10 µL template DNA). The following is how the cycling program was created: Taq enzyme activation/hold: 1 cycle at 95°C for 15 minutes; denaturation at 95°C for 20 seconds; annealing at 60°C for 20 seconds; and elongation at 72°C for 20 seconds.

Statistical analysis

The data was entered and analyzed with Microsoft Office 2017 Excel sheets and GraphPad Prism 8.4.3. Descriptive statistics for the data and variables are presented as frequencies and percentages.

RESULTS

Demographic and Clinical Characteristics of the Study Population

A total of 171 pathogenic bacterial strains were isolated from 219 clinical samples and tested for antibiotic resistance patterns. This study aimed to investigate the prevalence of resistant organisms and the efficiency of commonly used antibiotics against these infections. The investigation included 142 Gram-negative isolates. The majority of patients (30.7%) were over the age of 60, followed by those between 41 and 60 years (25.3%) and 19 to 40 years (22.7%), with a statistically significant relationship between age distribution and the examined result ($p = 0.021$). Males constituted 56.0% of the sample, while females made up 44.0%; however, this gender distribution was not statistically significant ($p = 0.141$). Regarding sample types, urine samples were the most frequently collected (34.7%), followed by blood (30.0%), wound swabs (22.0%), and respiratory samples (13.3%), showing a significant association ($p = 0.012$). Patients were admitted across various wards, with the highest proportion from General Medicine (30.7%) and Pediatric Ward (28.7%), while ICU admissions accounted for 16.0%. Ward type distribution was significantly associated with the outcome ($p = 0.018$). Among comorbidities, hypertension (30.0%) and diabetes (25.3%) were most common, while 41.3% of patients had no comorbid conditions. The presence of comorbidities showed a significant association ($p = 0.002$).

In terms of hospital stay duration, 38.0% of patients stayed for 7–14 days, 32.0% stayed more than 14

days, and 30.0% stayed for less than 7 days, with a significant association observed (p = 0.003). The majority of patients (80.0%) achieved full recovery, whereas 10.0% had recovery with complications, and 10.0% showed no improvement. Treatment outcomes were significantly associated with patient characteristics (p = 0.018). Empirical antibiotic therapy was more commonly used (72.0%) compared to targeted therapy (28.0%), with a significant association (p = 0.017). Although 27.3% of patients had hospital-acquired illnesses, this was not statistically significant (p = 0.141). [Table 1]

Table 1: Demographic Data of Patients (n = 142)

Demographic Variable	Category	Number of Patients (n)	Percentage (%)	p-value
Age	< 1 year	5	3.3%	0.021
	1-5 years	15	10.0%	
	6-18 years	12	8.0%	
	19-40 years	34	22.7%	
	41-60 years	38	25.3%	
	> 60 years	46	30.7%	
Gender	Male	84	56.0%	0.141
	Female	66	44.0%	
Sample Type	Blood	45	30.0%	0.012
	Urine	52	34.7%	
	Wound Swabs	33	22.0%	
	Respiratory Samples	20	13.3%	
Ward Type	ICU	24	16.0%	0.018
	General Medicine	46	30.7%	
	Surgery	27	18.0%	
	Obstetrics and Gynecology	10	6.7%	
	Pediatric Ward	43	28.7%	
Comorbidities	Diabetes	38	25.3%	0.002
	Hypertension	45	30.0%	
	Chronic Kidney Disease	15	10.0%	
	Cardiovascular Diseases	20	13.3%	
	COPD	8	5.3%	
	No comorbidity	62	41.3%	
Hospital Stay Duration (days)	< 7 days	45	30.0%	0.003
	7-14 days	57	38.0%	
	> 14 days	48	32.0%	
Outcome of Treatment	Full Recovery	120	80.0%	0.018
	Recovery with Complications	15	10.0%	
	No Improvement	15	10.0%	
Antibiotic Therapy Used	Empirical Therapy	108	72.0%	0.017
	Targeted Therapy	42	28.0%	
Hospital-acquired Infection	Yes	41	27.3%	0.141
	No	109	72.7%	

Clinical Presentation of Infections

The most prevalent clinical manifestation among the 142 isolates studied was fever, which occurred in 66.0% of cases and was statistically significant (p = 0.026). Other frequently reported symptoms included sepsis (24.7%), painful urination indicative of urinary tract infections (22.0%), and cough related to respiratory tract infections (18.7%). Chest pain (14.0%) and wound redness and swelling (10.7%) were also noted, reflecting respiratory and wound infections, respectively. Additionally, abdominal pain (9.3%) and diarrhea (6.7%) were reported in gastrointestinal infections. [Table 2] A small proportion of patients (5.3%) were identified as asymptomatic carriers, showing no overt signs of infection.

Table 2: Clinical Presentation of Infections

Clinical Presentation	Number of Cases (n)	Percentage (%)	p-value
Fever	99	66.0	0.026
Cough (for RTI)	28	18.7	
Painful urination (for UTI)	33	22.0	
Chest pain (for RTI)	21	14.0	
Diarrhea (for GI infections)	10	6.7	
Abdominal pain	14	9.3	
Wound redness and swelling	16	10.7	
Sepsis	37	24.7	
No symptoms (asymptomatic carrier)	8	5.3	

Distribution of Clinical Specimens and Gram-negative Isolates

A total of 219 clinical specimens were processed, from which 171 bacterial isolates were obtained. Among these, 142 isolates (83.0%) were Gram-negative, while the remaining 29 (17.0%) were Gram-positive. The highest number of specimens was obtained from wound swabs/pus (80), yielding

68 isolates, of which 56 (39.4%) were Gram-negative, making it the predominant source of Gram-negative bacteria. Urine samples contributed 58 specimens, resulting in 47 isolates, with 45 (31.7%) being Gram-negative. Sputum samples accounted for 20 specimens and yielded 17 isolates, out of which 16 (11.3%) were Gram-negative. Blood specimens (33 in total) produced 24 isolates, with 13 (9.2%) identified as Gram-negative. Body fluids, including cerebrospinal fluid (CSF) and others, contributed 28 specimens, yielding 15 isolates, of which 12 (8.5%) were Gram-negative. [Table 3] This distribution highlights the predominance of Gram-negative bacteria in clinical infections, particularly from wound, urine, and respiratory sources.

Table 3: Distribution of Clinical Specimens and Gram-negative Isolates

Type of Specimen	Number of Specimen	Number of Isolates (n)	Gram-negative Isolates (n)	Percentage (%)
Urine	58	47	45	31.7
Blood	33	24	13	9.2
Sputum	20	17	16	11.3
Wound Swab / Pus	80	68	56	39.4
Body Fluids (CSF, etc.)	28	15	12	8.5
Total	219	171	142	100

Antibiotic Resistance Rates Among Gram-negative Bacterial Isolates

Of the 142 Gram-negative bacterial isolates, 44 (31.0%) were resistant to one or more drugs. Klebsiella pneumoniae had the highest resistance rate, with 21 of 55 isolates (47.7%) demonstrating resistance. Klebsiella oxytoca also showed a notable resistance rate, with 11 of 37 isolates (25.0%) being resistant. Escherichia coli accounted for 39 isolates, of which 8 (18.2%) were resistant. In contrast, Pseudomonas aeruginosa showed a lower resistance rate, with only 1 out of 7 isolates (2.3%). Proteus mirabilis had 2 resistant isolates out of 3, contributing 4.5%, while Proteus vulgaris, with a single isolate, was resistant, contributing 2.3% to the overall resistance pool. [Table 4] These findings emphasize that Klebsiella species, particularly K. pneumoniae, are major contributors to antibiotic resistance among Gram-negative bacteria in this setting, underlining the need for continuous surveillance and targeted antimicrobial stewardship strategies.

Table 4: Antibiotic resistance rates of the isolated from Gram-negative bacteria

Bacterial Species	Number of Isolates (n)	Number of Resistant	Resistance Rate (%)
Escherichia coli	39	8	18.2
Klebsiella pneumoniae	55	21	47.7
Pseudomonas aeruginosa	7	1	2.3
Klebsiella oxytoca	37	11	25.0
Proteus mirabilis	3	2	4.5
Proteus vulgaris	1	1	2.3
Total	142	44	100

Antibiotic Resistance Patterns Among Gram-negative Bacterial Isolates

Of the 142 Gram-negative bacterial isolates tested, 44 (31.0%) were antibiotic-resistant. The most resistant isolates were reported in Klebsiella pneumoniae, with 21 out of 55 (47.7%) exhibiting resistance. Klebsiella oxytoca followed with 11 resistant isolates (25.0%), and Escherichia coli contributed 8 resistant strains (18.2%). Resistance was also noted in Pseudomonas aeruginosa (2.3%), Proteus mirabilis (4.5%), and Proteus vulgaris (2.3%). Escherichia coli (n=39) showed high resistance to Cefotaxime (76.9%), Ceftazidime/Avibactam (82.1%), Ciprofloxacin (74.4%), and Levofloxacin (71.8%). Resistance to carbapenem (Meropenem) was comparatively lower at 20.5%. Klebsiella pneumoniae (n=55) exhibited the highest resistance rates to Cefotaxime (87.3%), Ceftazidime/Avibactam (80.0%), Gentamicin (78.2%), and Ciprofloxacin (60.0%). Notably, resistance to Meropenem was 38.2%, reflecting concern for carbapenem resistance. Klebsiella oxytoca (n=37) showed a similar pattern, with high resistance to Cefotaxime (86.5%), Ceftazidime/Avibactam (83.8%), Ciprofloxacin (70.3%), and Gentamicin (70.3%). Pseudomonas aeruginosa (n=7) had moderate resistance to Ciprofloxacin and Cefotaxime (42.9%), with lower resistance to other agents. Importantly, no resistance to Meropenem or Trimethoprim/Sulfamethoxazole was reported. Proteus vulgaris (n=1) and Proteus mirabilis (n=3) showed 100% resistance to most tested antibiotics, although the small sample sizes limit broader interpretation. [Table 5] Overall, the results highlight a high frequency of multidrug resistance, particularly in E. coli and Klebsiella species, with significant resistance to important antibiotics including carbapenems, fluoroquinolones, and cephalosporins. To stop the emergence of resistant bacteria in clinical settings, these findings emphasize the critical need for stringent antimicrobial stewardship, regular resistance monitoring, and customized antibiotic policy.

Table 5: Resistance Pattern of Bacterial Species

Bacterial Species	E. coli (n=39)		K. pneumoniae (n=55)		P. aeruginosa (n=7)		Proteus vulgaris (n=1)		Proteus mirabilis (n=3)		Klebsiella oxytoca (n=37)	
Antibiotic	R (n)	R (%)	R (n)	R (%)	R (n)	R (%)	R (n)	R (%)	R (n)	R (%)	R (n)	R (%)
Ciprofloxacin (CIP)	29	74.4%	33	60.0%	3	42.9%	1	100%	3	100%	26	70.3%
Cefotaxime (CTX)	30	76.9%	48	87.3%	3	42.9%	1	100%	3	100%	32	86.5%
Gentamicin (GM)	18	46.2%	43	78.2%	2	28.6%	1	100%	2	66.7%	26	70.3%
Meropenem (MEM)	8	20.5%	21	38.2%	-	-	1	100%	-	-	10	27.0%
Amikacin (AN)	12	30.8%	26	47.3%	1	14.3%	1	100%	1	33.3%	14	37.8%
Piperacillin-Tazobactam (TZP)	11	28.2%	29	52.7%	1	14.3%	1	100%	1	33.3%	17	45.9%
Trimethoprim/sulfamethoxazole (SXT)	6	15.4%	12	21.8%	-	-	1	100%	-	-	6	16.2%
Levofloxacin (LVX)	28	71.8%	32	58.2%	2	28.6%	1	100%	2	33.3%	22	59.5%
Doxycycline (DOX)	11	28.2%	15	27.3%	-	-	1	100%	-	-	14	37.8%
Ceftazidime/Avibactam (CAZ)	32	82.1%	44	80.0%	2	28.6%	1	100%	2	33.3%	31	83.8%
Cefepime (FEP)	22	56.4%	35	63.6%	2	28.6%	-	-	2	33.3%	21	56.8%

Molecular Detection of Antibiotic Resistance Genes

Molecular detection of antibiotic resistance genes indicated the existence of blaOXA-51 and blaOXA-23 genes in various Gram-negative bacterial species that are related with carbapenem resistance. Among the species tested, Klebsiella pneumoniae (n=55) had the highest prevalence of resistance genes, with 37 isolates (67.3%) positive for blaOXA-51 and 38 isolates (69.1%) positive for blaOXA-23. Escherichia coli (n=39) showed 24 isolates (61.5%) positive for blaOXA-51 and 23 isolates (59.0%) for blaOXA-23. Pseudomonas aeruginosa (n=7) had 5 isolates (71.4%) positive for blaOXA-51 and 4 isolates (57.1%) for blaOXA-23, showing a high percentage positivity for blaOXA-51. Klebsiella oxytoca (n=37) showed moderate rates of resistance, with 21 isolates (56.8%) positive for blaOXA-51 and 19 isolates (51.4%) positive for blaOXA-23. Proteus mirabilis (n=3) exhibited 2 isolates (66.7%) positive for blaOXA-51, and 1 isolate (33.3%) for blaOXA-23. Finally, Proteus vulgaris (n=1) was 100% positive for blaOXA-51 but negative for blaOXA-23. [Table 6] These findings highlight the widespread presence of carbapenemase genes, particularly in Klebsiella, E. coli, and P. aeruginosa, reinforcing the importance of molecular diagnostics for early detection and control of multidrug-resistant organisms.

Table 6. Molecular Detection of Antibiotic Resistance Genes

Bacterial Species	blaOXA-51		blaOXA-23	
	Positive Isolates (n)	Percentage (%)	Positive Isolates (n)	Percentage (%)
E. coli (n=39)	24	61.5%	23	59.0%
K. pneumoniae (n=55)	37	67.3%	38	69.1%
P. aeruginosa (n=7)	5	71.4%	4	57.1%
Klebsiella oxytoca (n=37)	21	56.8%	19	51.4%
Proteus mirabilis (n=3)	2	66.7%	1	33.3%
Proteus vulgaris (n=1)	1	100.0%	-	-

Gene Amplification Among Isolates Using Real-Time PCR

The CFX96™ Real-Time PCR Detection System (BioRad) was used to detect the carbapenem resistance genes, blaOXA-51 and blaOXA-23. The Ct values for the positive PCR reactions ranged from 15 to 35 cycles, with lower Ct values indicating higher gene copy numbers. In Escherichia coli, blaOXA-51 was detected at 25.2 cycles and blaOXA-23 at 24.5 cycles. Klebsiella pneumoniae showed blaOXA-51 at 22.4 cycles and blaOXA-23 at 23.1 cycles, indicating relatively high gene presence. Pseudomonas aeruginosa had blaOXA-51 at 23.5 cycles and blaOXA-23 at 24.9 cycles. Klebsiella oxytoca exhibited blaOXA-51 amplification at 18.5 cycles, indicating a higher gene copy number, while blaOXA-23 appeared at 25.4 cycles. In Proteus mirabilis, blaOXA-51 was detected at 21.2 cycles and blaOXA-23 at 18.9 cycles, suggesting moderate gene presence. Lastly, Proteus vulgaris showed blaOXA-51 at 25.6 cycles, but no amplification of blaOXA-23. [Table 7] These results confirm the presence of carbapenemase genes in multiple bacterial species and demonstrate the variability in gene copy numbers, which can aid in assessing the resistance potential of these pathogens.

Table 7. Gene Amplification among Isolates

Bacterial Species	blaOXA-51	Ct Value (Cycle)	blaOXA-23	Ct Value (Cycle)
Escherichia coli	24	25.2	23	24.5
Klebsiella pneumoniae	37	22.4	38	23.1
Pseudomonas aeruginosa	5	23.5	4	24.9
Klebsiella oxytoca	21	18.5	19	25.4
Proteus mirabilis	2	21.2	1	18.9
Proteus vulgaris	1	25.6	-	-

DISCUSSION

The results of this investigation provide insight into the molecular mechanisms and antibiotic resistance patterns of Gram-negative bacteria isolated from clinical specimens at Government Theni Medical College and Hospital in Tamil Nadu. The findings revealed a large burden of antibiotic resistance, particularly among Klebsiella pneumoniae, Klebsiella oxytoca, and Escherichia coli. The resistance profiles found in this study are consistent with the global increase in antimicrobial resistance (AMR) in hospital settings, underscoring the importance of ongoing surveillance and targeted antimicrobial stewardship initiatives.

Antibiotic Resistance in Gram-negative Bacteria

The study discovered that among Gram-negative isolates, Klebsiella pneumoniae had the highest resistance rate, with 47.7% of its isolates resistant to at least one antibiotic. Klebsiella oxytoca also exhibited a significant resistance rate of 25.0%, while Escherichia coli had a moderate resistance rate of 18.2%. This result is comparatively lower than that of a Tanzanian study in which 85.2% of the cases were caused by Gram-negative bacteria.[15] Similar trends have been shown in research from India, where Klebsiella species with significant resistance to popular medicines including cephalosporins, fluoroquinolones, and carbapenems have been found to be important offenders in hospital-acquired infections (HAIs).[16] Consistent with our findings, Sodhi et al. (2020) found that Klebsiella pneumoniae was extremely resistant to beta-lactam antibiotics and carbapenems in a tertiary care hospital.[17]

The prevalence of carbapenem resistance, particularly in Klebsiella pneumoniae and Escherichia coli, is concerning, with 38.2% and 20.5% resistance to meropenem, respectively. These findings highlight the increasing threat of carbapenem-resistant enterobacteriaceae (CRE), which has been a subject of growing concern worldwide.[18] Our results are close to those of Datta et al. (2012) and Jaggi et al. (2019), who showed similar resistance rates in Klebsiella pneumoniae and Escherichia coli from Indian hospitals.[19, 20] The high carbapenem resistance is largely attributed to the production of carbapenemase enzymes, which is supported by our molecular detection of blaOXA-51 and blaOXA-23 genes in these isolates. Interestingly, Pseudomonas aeruginosa, which is known for its robust resistance mechanisms, showed a relatively low resistance rate of 2.3%. This is in contrast to studies conducted in other regions, such as those by Lee et al. (2024) and Khan et al. (2023), where Pseudomonas aeruginosa showed a high degree of resistance, particularly to fluoroquinolones and beta-lactams.[21,22] However, the absence of resistance to meropenem and trimethoprim-sulfamethoxazole in our investigation is a good finding, however additional monitoring is required to follow any changes in resistance patterns. The molecular detection of resistance genes revealed the ubiquitous presence of blaOXA-51 and blaOXA-23, which are linked to carbapenem resistance. These results are comparable to those of Rajan et al. (2023), who discovered that the blaOXA-51 gene was extremely frequent in Klebsiella pneumoniae isolates from hospital patients in India.[23] Our study also supports the assertion that carbapenemase-producing bacteria are a major threat in healthcare settings, particularly in regions where antibiotic use is poorly regulated.

Clinical Presentation and Demographics

According to the study population's clinical and demographic characteristics, the majority of patients were over 60, and the most common comorbidities were diabetes and hypertension. This is consistent with other research showing that multidrug-resistant infections are more common in older patients and those with underlying medical conditions.[24] The most common clinical presentation was fever (66%), followed by sepsis (24.7%) and urinary tract infections (22%). These findings correlate with the results of other studies from Indian hospitals, where fever and sepsis were commonly reported symptoms of Gram-negative bacterial infections, often associated with poor prognosis and prolonged hospital stays.[25] Additionally, the study discovered that the most often isolated organisms from urine, blood, and wound swabs were Klebsiella pneumoniae and Escherichia coli, indicating their role as important pathogens in bloodstream infections, surgical site infections, and urinary tract infections. The importance of specimen-specific antimicrobial susceptibility testing in guiding practical therapy is shown by the significant correlation between sample types and resistance outcomes.

Comparative Studies and Global Trends

A comparison with other studies demonstrates the global scope of AMR and the importance of tackling this issue. The US and Europe have seen alarmingly high levels of carbapenem resistance, mostly in Escherichia coli and Klebsiella pneumoniae. There have also been reports of colistin resistance.[26,27] Studies from Africa and Southeast Asia, including India, consistently report high levels of resistance to first-line antibiotics, with an increasing burden of carbapenem-resistant organisms.[28,29] However, our study, while reporting a high prevalence of resistance, also highlights the relatively lower rates of resistance in Pseudomonas aeruginosa compared to other regions, suggesting regional variation in AMR patterns. The identification of molecular markers of resistance, such as the blaOXA and blaNDM genes, is critical for improving diagnostic capabilities and infection control strategies. Molecular epidemiology studies like ours play a key role in identifying resistant strains, monitoring their spread, and informing antimicrobial stewardship policies.

CONCLUSION

In a tertiary care hospital in rural Tamil Nadu, this study discovered a notable frequency of multidrug-resistant Gram-negative bacterial infections, primarily *Klebsiella pneumoniae* and *Escherichia coli*. The predominance of carbapenemase genes like *blaOXA-51* and *blaOXA-23* is closely linked to the alarming widespread resistance to vital antibiotics like cephalosporins, fluoroquinolones, and carbapenems. In order to stop the development of resistant microorganisms, these results emphasize the vital necessity of regular molecular surveillance, efficient antimicrobial stewardship, and infection control procedures. Managing and reducing the issue of antimicrobial resistance in clinical settings requires strengthening local diagnostic skills and customizing empirical treatment based on regional resistance patterns. In order to effectively tackle AMR, geographical variations in resistance patterns further highlight the necessity of customized infection control tactics and localized surveillance.

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Ethical Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Government Theni Medical College & Hospital, Theni (Ref No: 1515/MEII/2024/53 dated 04.07.2024).

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