ORIGINAL ARTICLE

COMPARATIVE STUDY OF BACTERIAL ISOLATES FROM DIFFERENT CLINICAL SPECIMENS IN A TERTIARY CARE HOSPITAL

Soma Kanta Baral[1,2] ✉, Kailash Nath Acharya[1], Ram Krishna Shrestha[1], Deliya Paudel[1,2], Gita Shrestha[2], Himanshu Regmi[1,2], Upsal Bahadur Bajracharya[2], Muza Shrestha[2], Rajiv Nakarmi[2] and Eebaraj Simkhada[2]

[1]*Department of Laboratory Medicine, Manmohan Memorial Institute of Health Sciences, Soalteemode, Kathmandu*
[2]*Manmohan Memorial Medical College and Teaching Hospital, Kathmandu*

Received: October 25, 2025
Accepted: December 11, 2025
Published: December 15, 2025

✉ **Soma Kanta Baral,**
Associate Professor, Department of Laboratory Medicine, Manmohan Memorial Institute of Health Sciences, Soalteemode Kathmandu
Email: somabpkmch@gmail.com

https://doi.org/10.3126/jmmihs.v10i2.87416

How to Cite

Baral, S.K. et al. Comparative Study of Bacterial Isolates from Different Clinical Specimens in a Tertiary Care Hospital. Journal of Manmohan Memorial Institute of Health Sciences. 10, 2, 50–55. DOI:https://doi.org/10.3126/jmmihs.v10i2.87416.

ABSTRACT

Introduction: Antimicrobial resistance (AMR) is a critical global health threat, particularly in resource-limited settings. Continuous local surveillance of bacterial isolates and their resistance patterns is essential for guiding evidence-based antimicrobial therapy and infection control strategies. Consequently, the aim of this research is to characterize bacterial isolates from different clinical specimens in a tertiary care hospital and analyze their antimicrobial resistance patterns.

Method: A retrospective cross-sectional study was conducted at Manmohan Memorial Teaching Hospital, Kathmandu, from July 2024 to June 2025. A total of 15,525 clinical specimens including urine, sputum, pus, blood, and other body fluids, were processed using standard microbiological procedures. Bacterial identification was performed using colony morphology, Gram staining, and biochemical tests. Antimicrobial susceptibility testing (AST) was performed using the modified Kirby-Bauer disk diffusion method according to Clinical Laboratory Standards Institute (CLSI) 2023 guidelines. The Multiple Antibiotic Resistance (MAR) index was calculated for each isolate.

Result: Of 15,525 specimens processed, 1,475 (9.5%) showed significant bacterial growth. Escherichia coli (52.6%) predominated, followed by *Klebsiella pneumoniae* (18.3%) and *Staphylococcus aureus* (8.0%). *E. coli* demonstrated high resistance to cefixime (59.9%) and ceftazidime (73.3%), while S. aureus exhibited 51.6% methicillin resistance. Over 66% of isolates demonstrated a MAR index ≥0.2, indicating the bacteria originated from an environment where antibiotics are frequently used. Gram-negative organisms showed significantly higher resistance rates to third-generation cephalosporins and fluoroquinolones compared to Gram-positive organisms.

Conclusion: This study documents concerning levels of antimicrobial resistance among bacterial isolates at the tertiary care hospital, observing a predominance of isolates with a MAR index ≥0.2, multidrug-resistant Gram-negative bacilli, and methicillin-resistant Staphylococcus aureus (MRSA). These findings emphasize the urgent need for routine AMR surveillance, updated empirical treatment guidelines, and the implementation of hospital-based antimicrobial stewardship and infection control programs

Key words: Bacterial isolates; Antimicrobial resistance; MAR index; MRSA; *E. coli*; *Klebsiella pneumoniae; Pseudomonas aeruginosa*; Tertiary care hospital; Nepal

INTRODUCTION

Healthcare-associated infections remain a significant clinical burden, arising within healthcare environments and often transmitted via contaminated surfaces, medical devices, and healthcare workers. These infections pose serious risks, especially to immunocompromised patients, and frequently involve antimicrobial-resistant strains that complicate treatment and increase mortality rates[1].

Antimicrobial resistance (AMR) is now recognized as one of the foremost global public health threats, with far-reaching implications for disease management and healthcare costs. The World Health Organization (WHO) identifies AMR as one of the top ten global public health challenges facing humanity[2]. According to recent epidemiological data, bacterial AMR was linked to nearly 5 million deaths in 2019, with 1.27 million being directly attributed to resistant infections, particularly in low- and middle-income countries (LMICs) where access to effective antimicrobial therapy and diagnostic services remains limited[3]. Projections estimate that if left unchecked, AMR could claim 10 million lives annually by 2050 while causing significant economic damage up to US$3.4 trillion in annual gross domestic product (GDP) losses [4].

Resistance in major pathogens such as *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus*, and *Acinetobacter baumannii* is rising rapidly, threatening the efficacy of last-resort antibiotics including carbapenems and colistin[2,5]. The misuse and overuse of antibiotics in human medicine, animal husbandry, and agriculture have accelerated selection pressure for resistance. Simultaneously, inadequate infection prevention, poor sanitation, lack of timely diagnostics, and weak regulatory frameworks exacerbate this situation, particularly in developing regions [6, 7].

In LMICs like Nepal, AMR is intensified by limitations in diagnostic capabilities, fragmented surveillance systems, and frequent reliance on empirical therapy without laboratory confirmation. Such circumstances often lead to ineffective treatment and fuel further resistance development. Research suggests that although microbiological culture and sensitivity testing (CST) are vital for guiding antimicrobial use, they remain underutilized in clinical practice. More than 90% of hospitalized patients receive empirical antibiotics, and in many cases, only approximately 50% of therapies are adjusted after culture results become available[8]. This oversight hinders antimicrobial stewardship initiatives.

Despite global calls for antimicrobial stewardship, laboratory-based surveillance remains inconsistent and fragmented in many parts of the world. Diagnostic delays due to lack of infrastructure or resources often lead to inappropriate or delayed treatment, contributing to worse clinical outcomes[9]. Thus, AMR represents not only a biological threat but also a systemic failure involving diagnostics, treatment, regulation, and public health surveillance.

Monitoring the distribution of bacterial species across diverse clinical specimens (such as urine, blood, pus, and sputum) and their resistance patterns is indispensable for guiding effective treatment and limiting the spread of resistant

strains[10,11]. Clinical microbiology laboratories generate crucial data revealing both the burden of resistant pathogens and gaps in diagnostic and treatment approaches. Retrospective analyses of such data have proven valuable in evaluating resistance trends, monitoring infection control effectiveness, and identifying high-risk areas or populations[12].

This study aimed to conduct a comprehensive comparative analysis of bacterial isolates from different clinical specimens in a tertiary care hospital and characterize their antimicrobial resistance patterns, with the goal of enhancing local antimicrobial strategies and supporting broader initiatives against AMR.

METHODS

Study Design and Setting

A retrospective cross-sectional study was conducted at Manmohan Memorial Teaching Hospital (MMTH), Swoyambhu, Kathmandu, Nepal, from July 2024 to June 2025. The hospital is a 300-bed tertiary care facility serving both inpatient and outpatient populations from across Nepal.

Study Population and Samples

The study included all clinical specimens (urine, blood, sputum, pus, cerebrospinal fluid, and other body fluids) processed by the bacteriology laboratory during the study period. Specimens were collected from patients of all ages visiting the hospital. Inclusion criteria were all culture-positive specimens with significant bacterial growth. Exclusion criteria were contaminant cultures and repeated isolates from the same patient within 7 days.

Sample Collection and Processing

Clinical specimens were collected using standard aseptic techniques and transported to the microbiology laboratory within 2 hours of collection. Specimens were processed as follows:

Urine specimens: Inoculated onto Cystine Lactose Electrolyte Deficient (CLED) medium and incubated aerobically at 37°C for 24 hours.

Sputum, pus, and wound specimens: Inoculated onto Blood Agar, MacConkey Agar, and Chocolate Agar, incubated aerobically at 37°C for 24 hours.

Blood specimens: Inoculated into Brain Heart Infusion (BHI) broth and incubated aerobically at 37°C for 24 hours, with continued incubation for up to 72 hours if no growth was observed.

Chocolate agar plates: Incubated in 5-10% CO_2 incubator at 37°C for 24 hours.

Culture bottles showing positive growth were sub-cultured on Blood Agar, MacConkey Agar, and Chocolate Agar, and then incubated for 24 hours.

Bacterial Identification

Isolated bacteria were identified based on colony characteristics, morphological features, Gram staining, and biochemical tests. Bacteria were classified as Gram-positive or Gram-negative based on Gram reaction. Gram-negative bacteria were identified using biochemical tests including oxidase, catalase, indole, urea hydrolysis, citrate utilization, Triple Sugar Iron (TSI) agar, and motility tests. Gram-positive organisms were identified based on Gram reaction, hemolytic pattern, catalase reaction, and coagulase test. Staphylococcus aureus was confirmed by positive coagulase reaction.

Purity Confirmation

Purity plates were used to confirm aseptic technique and culture purity. A 4-hour inoculum was inoculated on one half of nutrient agar. After biochemical tests were completed, the other half was inoculated with the same organism. Following incubation at 37°C for 24 hours, matching growth on both halves confirmed maintained aseptic technique.

Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing was performed using the modified Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (MHA) following CLSI 2023 guidelines[13]. Three to five well-isolated colonies of the same morphological type were transferred to nutrient broth and incubated at 37°C for 2-4 hours until turbidity matched the 0.5 McFarland standards.

The bacterial suspension was evenly inoculated onto Mueller-Hinton Agar (MHA) plates using a sterile cotton swab by streaking the entire surface three times, rotating the plate approximately 60° between streaks to ensure uniform distribution. The inoculated plates were allowed to dry at room temperature for 10–15 minutes before placing antibiotic discs (HiMedia, India) on the surface with an inter-disc distance of at least 15 mm and a margin of 25 mm from the plate edge. The plates were then incubated aerobically at 37 °C for 16–18 hours. The diameter of inhibition zones around each antibiotic disc was measured in millimeters and interpreted according to CLSI zone size interpretative charts as Sensitive (S), Intermediate (I), or Resistant (R)[13]. But, Intermediate resistsnt was considered as resistant for the analysis.

Antibiotics tested included: Amoxycillin-Clavulanic acid, Amikacin, Cefixime, Cefotaxime, Cefoxitin, Ceftazidime, Ceftriaxone, Ciprofloxacin, Cotrimoxazole, Gentamicin, Levofloxacin, Meropenem, Nitrofurantoin, Piperacillin-Tazobactam, and Vancomycin.

Multiple Antibiotic Resistance (MAR) Index

The MAR index was calculated for each bacterial isolate using the formula:

MAR index = a/b

Where: a = number of antibiotics to which the isolate was resistant; b = total number of antibiotics tested for the isolate.

An MAR index ≥0.2 suggested that the bacterial isolates were derived from environments with frequent antibiotic exposure [14].

Data Analysis

Data were collected manually from culture reports and entered into a database. Analysis was performed using Excel and SPSS version 26. Descriptive statistics were used to present frequencies and percentages.

Ethical Considerations

Ethical approval was obtained from the Institutional Review Committee (NEHCO-IRC/081/100) of Manmohan Memorial Institute of Health Sciences. Permission was granted by the hospital administration. Patient confidentiality was maintained throughout the study, with all data de-identified prior to analysis.

RESULTS

Distribution of Specimens

A total of 15,525 clinical specimens were processed during the study period. As shown in Figure 1, the majority of the samples received were urine, accounting for 60% (n = 9,303) of the total sample. This was followed by blood samples (n = 3,062; 20%) and sputum samples (n = 1,838; 12%). Pus samples constituted a minor proportion of the collection (n = 343; 2%). The remaining 6% (n = 979) comprised various other specimen types, including body fluids, swabs, and stool.

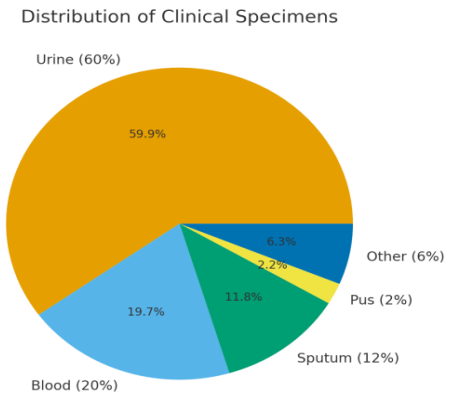

Figure 1: Distribution of Clinical Samples

Culture Positivity

Of the 15,525 specimens processed, 1,475 (9.5%) yielded positive bacterial growth. Among different specimen types, urine showed the highest positive culture rate at 1,024 (69.4%), followed by sputum 264 (17.9%), pus 98 (6.6%), other specimens 70 (4.7%), and blood 19 (1.3%).

Table 1: Culture Positivity Distribution

Specimen Type	Positive Cultures (n)	Percent Positives (%)
Urine	1,024	69.4
Sputum	264	17.9
Pus	98	6.6
Other	70	4.7
Blood	19	1.3
Total	1,475	100.0

Characteristics of the Study Population

Of 1,475 total positive cultures, 622 (42.16%) were from male patients and 853 (57.83%) were from female patients. The highest frequency was observed in the geriatric population (≥65 years), accounting for 387 (26.2%) patients. The majority of positive cultures were from outpatients (1,109; 75.2%) compared to inpatients (366; 24.8%). Urine specimens represented the largest proportion of positive cultures at 1,025 (69.5%).

Table 2: Characteristics of Study Population (n = 1,475)

Characteristic	Category	Number	Percent
Gender	Male	622	42.16
	Female	853	57.83
Age Group (years)	1–4	54	3.7
	5–14	40	2.7
	15–24	162	11.0
	25–34	298	20.2
	35–44	226	15.3
	45–54	161	10.9
	55–64	147	10.0
	≥65	387	26.2
Patient Type	Outpatient	1,109	75.2
	Inpatient	366	24.8

Frequency Distribution of Bacterial Isolates

Of the 1,475 positive cultures, *E. coli* predominated at 776 (52.6%), followed by *Klebsiella pneumoniae* 270 (18.3%), *Staphylococcus spp.* 118 (8.0%), *Pseudomonas aeruginosa* 114 (7.7%), *Enterococcus spp.* 43 (2.9%), *Enterobacter spp.* 30 (2.0%), *Citrobacter spp.* 25 (1.7%), *Burkholderia spp.* 23 (1.6%), *Acinetobacter spp.* 22 (1.5%), and *Proteus spp.* 14 (0.9%).

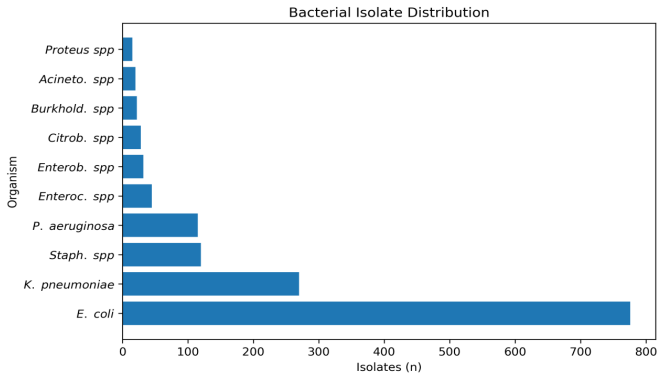

Figure 2: Distribution of Bacterial Isolates

Organism Distribution by Specimen Type

The distribution of bacterial isolates varied significantly across different clinical specimens. Among the isolates recovered from urine samples (n = 1,025), *Escherichia coli* was the predominant pathogen, accounting for 66.4% (n = 681) of the total. This was followed by *Klebsiella spp.* (14.3%), *Staphylococcus spp.* (6.0%), *Enterococcus spp.* (3.6%), and *Pseudomonas aeruginosa* (3.5%). In respiratory specimens (n = 264), *Klebsiella spp.* was the most frequently isolated organism (n = 99; 37.5%), followed by *P. aeruginosa* (n = 63; 23.9%) and *E. coli* (n = 45; 17.0%). *Acinetobacter spp.* and *Burkholderia spp.* were also identified, accounting for 5.7% and 4.9% of the isolates, respectively. Analysis of isolates from pyogenic infections (n = 98) revealed that *Staphylococcus aureus* was the major causative agent, representing 51.0% (n = 50) of the cases. Gram-negative bacteria, including E. coli (25.5%), Klebsiella spp. (9.2%), and P. aeruginosa (5.1%), were less frequent. In other clinical specimens (n = 70), *Staphylococcus spp.* was the most common isolate (n = 26; 37.1%). Other notable pathogens included *E. coli* (28.6%), P. aeruginosa (11.4%), and *Klebsiella pneumoniae* (11.4%).

Antimicrobial Resistance Patterns in Gram-Negative Organisms

Enterobacteriaceae (E. coli and K. pneumoniae): Both Escherichia coli (n=776) and Klebsiella pneumoniae (n=270) exhibited high rates of resistance to third-generation cephalosporins, particularly ceftazidime (73.3% and 71.5%, respectively) and cefixime (>56%). Resistance to amoxycillin-clavulanic acid was notably high in both organisms (>63%). Fluoroquinolone resistance ranged from 45% to 52%. Meropenem resistance was observed in 15.7% of E. coli isolates, whereas K. pneumoniae showed varying susceptibility profiles.

Non-fermenting Gram-negative Bacilli: Pseudomonas aeruginosa (n=114) demonstrated significant resistance to ceftazidime (48.2%) and piperacillin-tazobactam (42.1%). Notably, carbapenem resistance (meropenem) was higher in P. aeruginosa (28.9%) compared to the Enterobacteriaceae. Acinetobacter spp. (n=22) emerged as the most resistant pathogen group, exhibiting high resistance rates to cephalosporins (59 - 68%), fluoroquinolones (63.6%), and piperacillin-tazobactam (59.1%). Meropenem resistance in Acinetobacter spp. was alarming at 40.9%.

Table 3: Distribution of Bacterial Isolates by Clinical Specimen Type (n = 1,475)

Organism	Urine (n=1,025)	Sputum (n=264)	Pus (n=98)	Blood (n=19)	Other (n=70)	Total
E. coli	681 (66.4%)	45 (17.0%)	25 (25.5%)	3 (15.8%)	20 (28.6%)	776 (52.6%)
Klebsiella pneumoniae	147 (14.3%)	99 (37.5%)	9 (9.2%)	2 (10.5%)	8 (11.4%)	270 (18.3%)
Staphylococcus spp.	62 (6.0%)	12 (4.5%)	50 (51.0%)	1 (5.3%)	26 (37.1%)	118 (8.0%)
Pseudomonas aeruginosa	36 (3.5%)	63 (23.9%)	5 (5.1%)	1 (5.3%)	8 (11.4%)	114 (7.7%)
Enterococcus spp.	37 (3.6%)	2 (0.8%)	2 (2.0%)	1 (5.3%)	1 (1.4%)	43 (2.9%)
Acinetobacter spp.	3 (0.3%)	15 (5.7%)	2 (2.0%)	2 (10.5%)	0 (0.0%)	22 (1.5%)
Other organisms	59 (5.8%)	28 (10.6%)	5 (5.1%)	9 (47.4%)	7 (10.0%)	132 (8.9%)

Table 4: Antibiotic Resistance Profile of Gram-Negative Isolates

Antibiotic	*E. coli (n=776)*	*Klebsiella spp. (n=270)*	*Pseudomonas (n=114)*	*Acinetobacter (n=22)*
Beta-lactams				
Amoxycillin–Clavulanic Acid	65.3%	63.7%	—	59.1%
Cefixime	59.9%	56.3%	—	—
Cefotaxime	58.2%	54.8%	52.6%	68.2%
Ceftazidime	73.3%	71.5%	48.2%	59.1%
Piperacillin–Tazobactam	38.5%	35.2%	42.1%	59.1%
Meropenem	15.7%	12.0%	28.9%	40.9%
Fluoroquinolones				
Ciprofloxacin	50.0%	47.5%	35.1%	63.6%
Levofloxacin	48.0%	45.0%	32.0%	59.1%
Aminoglycosides				

Table 5: Antibiotic Resistance Profile of Gram-Positive Isolates

Antibiotic	*Staphylococcus aureus* (n=118)	*Enterococcus spp.* (n=43)
Beta-lactams		
Cloxacillin	51.6%	—
Ampicillin	—	65.1%
Piperacillin–Tazo-bactam	22.0%	18.2%
Fluoroquinolones		
Ciprofloxacin	38.2%	77.7%
Levofloxacin	35.6%	74.4%
Glycopeptides		
Vancomycin	0.0%	2.3%
Aminoglycosides		
Gentamicin	19.2%	79.1%
Other		
Cotrimoxazole	46.5%	—
Nitrofurantoin	12.7%	40.0%

Antimicrobial Resistance Patterns in Gram-Positive Organisms

Among the Staphylococcus aureus isolates (n=118), resistance to cloxacillin was observed in 51.6% (n=61), identifying these strains as Methicillin-Resistant Staphylococcus aureus (MRSA). Resistance to non-beta-lactam antibiotics was also substantial, with 46.5% of isolates resistant to cotrimoxazole and 38.2% to ciprofloxacin. Gentamicin resistance was relatively lower at 19.2%. Notably, all S. aureus isolates (100%) remained fully susceptible to vancomycin.

Enterococcus spp. (n=43) exhibited high rates of resistance to aminoglycosides and fluoroquinolones, with 79.1% resistant to gentamicin and 77.7% to ciprofloxacin. Resistance to nitrofurantoin was observed in 40.0% of cases. Piperacillin-tazobactam showed the highest susceptibility among the tested antibiotics, with a resistance rate of 18.2%.

Multiple Antibiotic Resistance Index

The Multiple Antibiotic Resistance (MAR) index was calculated to evaluate the health risk of the environments from which the organisms were isolated. The highest mean MAR index was recorded in *Acinetobacter spp.* (0.42 ± 0.13), followed by E. coli (0.38 ± 0.15) and K. pneumoniae (0.36 ± 0.14). *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed comparatively lower mean indices of 0.31 ± 0.12 and 0.29 ± 0.11, respectively. Notably, over 66% of all tested isolates demonstrated a MAR index ≥0.2. This threshold (>0.2) suggests that a majority of the isolates originated from a high-risk source of contamination where antibiotics are frequently used.

Table 6: Multiple Antibiotic Resistance (MAR) Index of Bacterial Isolates

Organism	n	Mean MAR Index	SD	Range	% with MAR ≥0.2
E. coli	776	0.38	±0.15	0.07–0.73	74.5%
Klebsiella pneumoniae	270	0.36	±0.14	0.07–0.67	72.2%
Staphylococcus aureus	118	0.31	±0.12	0.07–0.60	65.3%
Pseudomonas aeruginosa	114	0.29	±0.11	0.07–0.53	61.4%
Acinetobacter spp.	22	0.42	±0.13	0.20–0.73	86.4%
Total	1,475	0.35	±0.14	0.07–0.73	66.4%

DISCUSSIONS

This retrospective study of 15,525 clinical specimens from a tertiary care hospital in Kathmandu documents significant rates of antimicrobial resistance among bacterial pathogens. The overall culture positivity rate of 9.5% is consistent with previous reports from similar settings in South Asia [15, 16].

The predominance of *E. coli* (52.6%) aligns with global epidemiology, where this organism remains the most common cause of community-acquired urinary tract infections (UTIs) and represents a significant proportion of nosocomial infections[17,18]. The second most common pathogen, *Klebsiella pneumoniae* (18.3%), has emerged as a major healthcare-associated pathogen, particularly in respiratory tract infections and bloodstream infections [19].

The distribution of pathogens by specimen type follows expected patterns. *E. coli* dominated urinary isolates (66%), consistent with its role as the primary uropathogen[17]. *Klebsiella spp.* and *Pseudomonas aeruginosa* predominated in respiratory specimens, reflecting common causative agents of ventilator-associated pneumonia and nosocomial respiratory tract infections[20,21]. *Staphylococcus aureus* was the leading cause of pyogenic infections (51%), consistent with its role in skin and soft tissue infections, abscesses, and wound infections[22].

The resistance rates observed in this study are alarming and mandate urgent action. The 73.3% resistance of *E. coli* to ceftazidime and 59.9% resistance to cefixime represent critical threats to empirical treatment efficacy, as third-generation cephalosporins are commonly used empirically for serious infections in resource-limited settings[23]. High resistance to fluoroquinolones (48-52%) further limits treatment options, as fluoroquinolones are frequently used in outpatient settings.

The 51.6% methicillin resistance rate among *Staphylococcus aureus* is concerning and suggests a high prevalence of MRSA in this hospital. MRSA infections are associated with increased treatment failures, longer hospital stays, and higher mortality rates [24]. The finding that MRSA susceptibility to vancomycin remains 100% is reassuring and indicates that vancomycin remains a reliable treatment option for serious MRSA infections.

The high resistance of *Enterococcus spp.* to gentamicin (79.1%) and ciprofloxacin (77.7%) reflects the intrinsic resistance mechanisms of enterococci and highlights the challenges in treating enterococcal infections, particularly in cases of resistance to multiple drug classes [25].

The finding that over 66% of isolates had a MAR index ≥0.2 is highly concerning and indicates widespread multidrug resistance in the hospital environment. The highest MAR indices were observed in *Acinetobacter spp.* (0.42±0.13) and *E. coli* (0.38±0.15), organisms known to accumulate and transfer resistance mechanisms [26]. High MAR indices correlate with increased treatment failures and suggest that these organisms have been exposed to multiple antimicrobial agents, likely reflecting widespread inappropriate antibiotic use.

Interestingly, sputum and blood specimens showed different resistance patterns compared to urinary specimens. Respiratory isolates (*Klebsiella and Pseudomonas*) generally showed higher resistance rates to beta-lactams compared to urinary E. coli, potentially reflecting different ecological niches and selective pressures within the hospital[27, 28]. This suggests that empirical treatment regimens may need to be tailored based on specimen type and suspected source of infection [29].

Previous studies from tertiary care hospitals in Nepal documented similar resistance patterns. A study in hospitals of Kathmandu reported high prevalence of MRSA and fluoroquinolone resistance in *Staphylococcus aureus* [30, 31]. Studies from other South Asian countries (Bangladesh, India) have documented comparable resistance rates and pathogen distributions, suggesting that this resistance pattern represents a regional problem requiring coordinated responses [32, 33].

Limitations

This study was limited to a single tertiary care hospital in Kathmandu, which may not be entirely representative of other regions in Nepal. The study did not include analysis of antibiotic consumption patterns or correlation with clinical outcomes. Additionally, some organisms were not further speciated (e.g., *Enterobacter, Citrobacter*), which may have limited clinical interpretation.

CONCLUSION

This study highlights a critical burden of antimicrobial resistance in a tertiary care setting in Nepal, where over two-thirds (66%) of bacterial isolates exhibited a Multiple Antibiotic Resistance (MAR) index ≥0.2, indicating high antibiotic selection pressure. Escherichia coli, Klebsiella pneumoniae, and Methicillin-resistant Staphylococcus aureus (MRSA) were the predominant pathogens, demonstrating widespread resistance to essential therapeutics, particularly third-generation cephalosporins and fluoroquinolones. These findings suggest that current empirical treatment guidelines may need revision to align with local resistance patterns.

REFERENCES

1. World Health Organization. Healthcare-associated infections: A global challenge. WHO Policy Brief. 2022.
2. World Health Organization. Global antimicrobial resistance and use surveillance system (GLASS) report 2023. WHO; 2023.
3. Murray CJ, Ikuta KS, Sharara F, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022; 399(10325):629-655.
4. Antimicrobial resistance: accelerating national and global responses. WHO strategic and operational priorities to address drug-resistant bacterial infections in the human health sector, 2025–2035. WHO; 2024.
5. Tacconelli E, Carrara E, Savoldi A, et al. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. WHO; 2017.

6. Laxminarayan R, Duse A, Wattal C, et al. Antibiotic resistance—the need for global solutions. Lancet Infect Dis. 2013; 13(12):1057-1098.
7. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health. 2015; 109(7):309-318.
8. Baig AN, Guddeti PK, Verma BS, Wagh KB. Utility of culture and antibiotic sensitivity testing to combat antimicrobial resistance among admitted patients in a tertiary care hospital. Int J Sci Res. 2015; 4(3):2277-8179.
9. Okeke IN, Peeling RW, Goossens H, et al. Diagnostics as essential tools for containing antibacterial resistance. Drug Resist Updates. 2011; 14(4):295-306.
10. Abebe M, Tadesse S, Meseret G, Derbie A. Type of bacterial isolates and antimicrobial resistance profile from different clinical samples at a Referral Hospital, Northwest Ethiopia: five years data analysis. BMC Res Notes. 2019; 12(1):1-6.
11. Mk S, Sk M, Jb S, et al. Nosocomial bacterial infection and antimicrobial resistant pattern in a tertiary care hospital in Nepal. J Inst Med Nepal. 2014; 36(3):38-48.
12. Ventola CL. The antibiotic resistance crisis: Part 1: Causes and threats. P T. 2015; 40(4):277-283.
13. Clinical and Laboratory Standards Institute (CLSI). M100 Performance standards for antimicrobial susceptibility testing. 33rd ed. CLSI; 2023.
14. Krumperman PH. Multiple antibiotic resistance indexing of Escherichia coli to identify high-risk sources of fecal contamination of foods. Appl Environ Microbiol. 1983; 46(1):165-170.
15. Chaudhary BR, Malla KK, Poudel S, Jha BK. Study of antibiotic susceptibility among bacterial isolates in neonatal intensive care unit of a tertiary care hospital: a descriptive cross-sectional study. J Nepal Med Assoc. 2020; 58(231):893-899.
16. Karn M, Bhargava DI, Dhungel B, et al. The burden and characteristics of nosocomial infections in an intensive care unit: a cross-sectional study of clinical and nonclinical samples at a tertiary hospital of Nepal. Int J Crit Illn Inj Sci. 2021; 11(4):236-243.
17. Foxman B. The epidemiology of urinary tract infection. Nat Rev Urol. 2010; 7(12):653-660.
18. Gupta K, Hooton TM, Naber KG, et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis. 2011; 52(5):e103-e120.
19. Cao X, Xu X, Zhang Z, et al. Molecular characterization of clinical multidrug-resistant Klebsiella pneumoniae isolates. Ann Clin Microbiol Antimicrob. 2014; 13(1):1-8.
20. Kollef MH, Shorr A, Tabak YP, et al. Epidemiology and outcomes of health-care-associated pneumonia: results from a large US database of culture-positive pneumonia. Chest. 2005; 128(6):3854-3862.
21. Kollef MH. The prevention of ventilator-associated pneumonia. N Engl J Med. 1999; 340(8):627-634.
22. David MZ, Daum RS. Community-associated methicillin-resistant Staphylococcus aureus: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev. 2010; 23(3):616-687.
23. Gajdcs M, Bőrk M, Lázár A, et al. Comparative epidemiology and resistance trends of common urinary pathogens in a tertiary-care hospital: a 10-year surveillance study. Medicina (Kaunas). 2019; 55(7):357.
24. Holmes AH, Moore LSP, Sundsfjord A, et al. Understanding the mechanisms and drivers of antimicrobial resistance. Lancet. 2016; 387(10014):176-187.
25. Adhikari RP, Shrestha S, Barakoti A, et al. Antimicrobial susceptibility pattern of Enterococcus species isolated from various clinical specimens in a tertiary care hospital, Kathmandu, Nepal. Nepal Med Coll J. 2018; 20(4):173-177.
26. Blair JMA, Webber MA, Baylay AJ, et al. Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol. 2015; 13(1):42-51.
27. Parajuli RP, Bharati N, Bhandari S, Patel DK, Neupane A, Ansari Z, et al. Antibiotic resistance pattern of bacteria isolated from clinical specimens: a hospital-based cross-sectional study in Kathmandu, Nepal. Nepal Med Coll J. 2024; 26(2):132-7.
28. Baral SK, Dhakal A, Timilsina RP, Manandhar KD, Poudel P. Phenotypic insights into beta-lactamase-mediated multidrug resistance in Escherichia coli clinical isolates. J Manmohan Mem Inst Health Sci. 2024;10(1):51–4. doi: 10.3126/jmmihs.v10i1.77748.
29. Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. Infectious Diseases Society of America 2023 Guidance on the Treatment of Antimicrobial Resistant Gram-Negative Infections. Clin Infect Dis. 2023; 77(4):e157-e212.
30. Baral SK, et al. Bacterial isolates and antibiogram profile in clinically suspected otitis media patient. Acta Scientific Microbiology. 2024; 7(12):78-84.
31. Thapa RB, Shrestha S, Adhikari P, Shrestha R. Antibiotic resistance patterns in uropathogens: insights from a Nepalese tertiary care setting. Ther Adv Infect Dis. 2025; 12:20499361251339384.
32. Poudyal N, Begum F, Sujan MJ, et al. Bacterial profile and antimicrobial resistance pattern from different clinical specimens at Uttara Adhunik Medical College Hospital, Dhaka. J Clin Diagn Res. 2025; 19(3):DC01-DC05.
33. Biswas R, Rabbani R, Ahmed HS, et al. Antibiotic sensitivity pattern of urinary tract infection at a tertiary care hospital. Bangladesh Crit Care J. 2014; 2(1):21-24.

ACKNOWLEDGEMENTS

We thank the clinical microbiology laboratory staff and Manmohan Memorial Teaching Hospital administration for providing access to data and laboratory facilities.

COMPETING INTERESTS

All the authors declare no competing interest