ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26nov601

Prevalence, Bacterial Etiology and Antimicrobial Susceptibility Profiles in Hospitals

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Publication Date: 2026/11/18

Abstract: Antimicrobial resistance (AMR) threatens clinical medicine globally, compromising therapeutic efficacy and increasing mortality in resource-constrained sub-Saharan health settings. This study focused strictly on determining the overall prevalence, bacterial species distribution, specimen-specific isolation rates, and antimicrobial susceptibility testing (AST) profiles among hospitalized patients at Kirehe District Hospital, Eastern Province, Rwanda. A total of N = 50 hospitalized patients with validated bacterial culture and AST records were evaluated using a structured clinical microbiology extraction instrument. All data were analyzed using IBM SPSS Statistics version 28.0. Exact binomial 95% confidence intervals (CIs) were computed for prevalence estimates, alongside frequency distributions for isolated bacterial species and antibiotic resistance patterns across antimicrobial classes. The overall laboratory-confirmed AMR prevalence rate among isolated pathogens was 60.0% (n = 30/50; 95% CI: 46.2%–73.8%). Gram-negative Enterobacterales constituted the majority of bacterial isolates (56.0%, n = 28/50), led by Escherichia coli (36.0%, n = 18) and Klebsiella pneumoniae (20.0%, n = 10), while Gram-positive pathogens were dominated by Staphylococcus aureus (26.0%, n = 13). Resistance rates were highest among K. pneumoniae (70.0%, n = 7/10) and E. coli (66.7%, n = 12/18). High non-susceptibility was observed against first- and second-line empiric agents, including ampicillin (88.9%), trimethoprimsulfamethoxazole (80.0%), and ceftriaxone (64.3%), whereas carbapenems (imipenem, 92.0% susceptible) and aminoglycosides (gentamicin, 74.0% susceptible) demonstrated superior in-vitro activity. Multidrug resistance (MDR), defined as non-susceptibility to ≥ 1 agent in ≥ 3 antimicrobial categories, was documented in 44.0% (n = 22/50) of all isolates. These findings demonstrate a substantial burden of resistant and MDR bacterial pathogens in rural district hospital inpatients, highlighting the critical necessity for routine diagnostic microbiology surveillance and localized hospital antibiograms to guide empiric prescribing protocols.

Keywords: *Antimicrobial Resistance, AMR Prevalence, Pathogen Etiology, Susceptibility Profiles, Multidrug Resistance, Kirehe District Hospital, Rwanda.*

How to Cite: Dr. Jean de Dieu Nduwayezu; John Peter Ndikubwimana (2026) Prevalence, Bacterial Etiology and Antimicrobial Susceptibility Profiles in Hospitals. *International Journal of Innovative Science and Research Technology*, 11(11), 597-603. https://doi.org/10.38124/ijisrt/26nov601

I. INTRODUCTION

Antimicrobial resistance (AMR) represents one of the most urgent threats to global public health and modern healthcare delivery in the 21 st century (WHO, 2026). The global surge in bacterial pathogens resistant to conventional antibacterial agents threatens to render common infectious diseases incurable, compromise invasive surgical interventions, and dramatically increase healthcare-associated morbidity and mortality (Murray et al., 2022). The World Health Organization (WHO) Global Antibiotic Resistance Surveillance System (GLASS) report synthesized data across 23 million bacteriologically confirmed infections worldwide, establishing that approximately 16.7% (one in six) of common bacterial infections globally exhibited resistance to standard antibiotic treatments (WHO, 2025b).

Sub-Saharan Africa bears a disproportionate burden of AMRrelated mortality, driven by a high baseline infectious disease incidence coupled with structural healthcare challenges (Bunduki et al., 2024). In many regional health facilities, routine microbiological culture and antimicrobial susceptibility testing (AST) remain underutilized due to diagnostic infrastructure constraints, financial barriers, and reagent supply bottlenecks (Katakweba et al., 2023). Consequently, patient management in sub-Saharan African hospitals relies heavily on broad-spectrum empiric antimicrobial therapy, which can inadvertently accelerate local selection pressure and propagate multidrug-resistant (MDR) strains (Laxminarayan et al., 2023).

In Rwanda, the Ministry of Health has prioritized AMR surveillance through the implementation of the Strategic

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26nov601

Action Plan on Antimicrobial Resistance (2023–2027) (Ministry of Health Rwanda, 2023). Epidemiological investigations conducted at tertiary referral centers, notably the University Teaching Hospital of Kigali (CHUK), have provided valuable baseline data on resistance patterns among bloodstream and surgical site isolates (Habyarimana et al., 2021; Munyemana et al., 2025). However, microbiological data derived from urban referral institutions cannot be directly extrapolated to rural district hospital settings.

District hospitals operate within unique ecological and operational contexts, serving rural populations with distinct agricultural exposures, localized healthcare-seeking pathways, and specific empiric prescription practices. Kirehe District Hospital, situated in the Eastern Province of Rwanda, functions as the secondary referral facility for a rural population of over 340,000 residents across 16 health center catchment areas. Prior to this study, baseline empirical data detailing the exact prevalence of laboratory-confirmed AMR, bacterial species distributions, specimen isolation yields, and drugspecific susceptibility profiles among hospitalized patients at Kirehe District Hospital were absent.

Therefore, this manuscript focuses strictly on addressing this empirical gap by quantifying the baseline prevalence of laboratoryconfirmed AMR, defining the bacterial etiology across inpatient clinical wards, evaluating specimen-specific pathogen isolation distributions, and establishing comprehensive antibiotic susceptibility and non-susceptibility profiles. (Note: Associated clinical and sociocultural determinants collected during the parent project are segregated into dedicated sister manuscripts).

II. METHODOLOGY

➢ *Study Design and Investigation Setting*

A hospital-based descriptive cross-sectional microbiological investigation was conducted at Kirehe District Hospital, located in Kirehe District, Eastern Province, Rwanda. The facility maintains a 200-bed inpatient capacity distributed across four core clinical departments: Internal Medicine, General Surgery, Pediatrics, and Obstetrics & Gynecology (OB/GYN). The hospital operates a functional clinical microbiology laboratory equipped to perform standard bacterial culture, Gram staining, biochemical identification, and disc diffusion susceptibility testing.

➢ *Target Population and Inclusion Criteria*

The target population comprised all patients admitted to the inpatient wards of Kirehe District Hospital during the study period (October through November 2026). To be eligible for inclusion in this prevalence analysis, patient records were required to satisfy the following strict criteria:

- Formal inpatient admission status of ≥ 24 hours in any clinical ward.
- Complete clinical microbiology diagnostic records showing a positive bacterial culture isolate.
- Validated antimicrobial susceptibility testing (AST) results available for a panel ofstandard antibacterial agents.
- Documented clinical specimen source (e.g., blood, urine, wound swab, sputum, or body fluids).

➢ *Sampling Strategy and Cohort Size*

A non-probability convenience sampling strategy was utilized to enroll eligible patients during the study period. Because routine microbiology cultures in district hospitals are selectively ordered for patients presenting with severe, recurrent, or treatment-refractory infections, all consecutive inpatient records meeting the complete culture and AST criteria (N = 50) were extracted. This total sample size of 50 validated clinical microbiology records provided an immediate, high-fidelity baseline dataset to compute facility-level prevalence and resistance proportions.

➢ *Laboratory Diagnostic Protocols and Quality Control*

Clinical specimens were collected by ward clinical staff using standard aseptic techniques and delivered immediately to the microbiology laboratory. Specimen processing followed standard bacteriological procedures:

- *Urine:* Cultured on CLED (Cysteine-Lactose-Electrolyte-Deficient) agar and MacConkey agar using calibrated loops (significant bacteriuria defined as $\geq 10^5$ CFU/mL).
- *Blood:* Cultured in brain heart infusion (BHI) broth bottles, subcultured onto Blood Agar, Chocolate Agar, and MacConkey Agar upon detection ofturbidity.
- *Wound Swabs / Pus:* Inoculated onto Blood Agar, Mannitol Salt Agar (for staphylococci isolation), and MacConkey Agar.
- *Sputum / Respiratory Exudates:* Gram stained for quality screening (< 10 epithelial cells/HPF) and cultured on Blood, Chocolate, and MacConkey plates.

Bacterial species identification was confirmed through colony morphology, Gram stain reaction, catalase and oxidase testing, and standard biochemical panels (indole, citrate, urease, triple sugar iron, coagulase, and motility testing) (CLSI, 2024). Quality control was maintained using standard reference strains: *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923.

➢ *Antimicrobial Susceptibility Testing (AST)*

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar plates, standardized to 0.5 McFarland turbidity standards, following Clinical and Laboratory Standards Institute (CLSI M100, 34th edition) guidelines (CLSI, 2024). Inhibition zone diameters were measured after 18–24 hours of aerobic incubation at 35°C–37°C. Antibiotic panels tested included:

- *Penicillins / Beta-lactams:* Ampicillin (10 µg), Amoxicillinclavulanic acid (20/10 µg).
- *Cephalosporins:* Ceftriaxone (30 µg), Cefotaxime (30 µg), Ceftazidime (30 µg).
- *Carbapenems:* Imipenem (10 µg), Meropenem (10 µg).

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- *Aminoglycosides:* Gentamicin (10 µg), Amikacin (30 µg).
- *Fluoroquinolones:* Ciprofloxacin (5 µg).
- *Folate Pathway Inhibitors:* Trimethoprim-sulfamethoxazole (1 .25/23.75 µg).
- *Glycopeptides / Macrolides:* Vancomycin (30 µg), Erythromycin (15 µg - Gram-positives).

➢ *Operational Definitions for Prevalence Metrics*

For analytical classification:

- AMR Positive (Resistant): A bacterial isolate classified as nonsusceptible (Intermediate [I] or Resistant [R]) to at least one tested antimicrobial agent in the laboratory panel.
- AMR Negative (Susceptible): An isolate demonstrating full susceptibility (S) to all tested antimicrobial agents.
- Multidrug Resistance (MDR): Defined per international consensus guidelines as non-susceptibility to at least one agent in three or more distinct antimicrobial categories (Magiorakos et al., 2012).

➢ *Statistical Analysis*

Microbiological data were coded, validated, and analyzed using IBM SPSS Statistics version 28.0. Overall AMR prevalence was calculated as the proportion of AMR Positive patients out of total enrolled inpatients (N = 50), with exact 95% binomial confidence intervals. Frequency distributions and cross-tabulations were generated to describe pathogen etiology, specimen source yield, ward-specific pathogen burden, and antibiotic non-susceptibility percentages.

III. RESULTS AND FINDINGS

➢ *Baseline Inpatient & Specimen Characteristics*

The study evaluated N = 50 hospitalized patients with validated microbiology records. The patient sample comprised 54.0% females (n = 27) and 46.0% males (n = 23), with a mean age of 38.4 ± 15.2 years. Clinical distribution across wards showed the highest representation from Internal Medicine (44.0%, n = 22), followed by General Surgery (30.0%, n = 15), Pediatrics (14.0%, n = 7), and OB/GYN (12.0%, n = 6).

Clinical specimens yielding positive bacterial growth were categorized across five specimen types: Urine (36.0%, n = 18), Wound Swabs/Pus (34.0%, n = 17), Blood (14.0%, n = 7), Sputum (10.0%, n = 5), and Body Fluids (6.0%, n = 3). Table 1 outlines the complete specimen source yield and corresponding resistance counts.

Table 1 Specimen Source Yield & AMR Distribution (N = 50)

Specimen Type	Total Isolates n (%)	AMR Positive n (%)	AMR Negative n (%)
Urine	18 (36.0%)	12 (66.7%)	6 (33.3%)
Wound Swab / Pus	17 (34.0%)	11 (64.7%)	6 (35.3%)
Blood Culture	7 (14.0%)	4 (57.1%)	3 (42.9%)
Sputum	5 (10.0%)	2 (40.0%)	3 (60.0%)
Body Fluids (Aschit/CSF)	3 (6.0%)	1 (33.3%)	2 (66.7%)
Total Sample	**50 (100.0%)**	**30 (60.0%)**	**20 (40.0%)**

➢ *Overall AMR Prevalence Rate*

Out of the 50 analyzed inpatient microbiology records, 30 isolates demonstrated resistance to at least one tested antibacterial agent, yielding an overall baseline AMR prevalence rate of 60.0% (95% CI: 46.2% – 73.8%). The remaining 20 isolates (40.0%, 95% CI: 26.2% – 53.8%) were fully susceptible to all tested agents in the Ampicillin Penicillin 36 4 (11 .1 %) 2 (5.6%) facility panel.

➢ *Bacterial Etiology and Species Distribution*

Gram-negative bacteria accounted for 66.0% (n = 33/50) of all clinical isolates, whereas Gram-positive species comprised 34.0% (n = 17/50). Five distinct bacterial species were identified: *Escherichia coli* (36.0%, n = 18), *Staphylococcus aureus* (26.0%, n = 13), *Klebsiella pneumoniae* (20.0%, n = 10), *Pseudomonas aeruginosa* (10.0%, n = 5), and *Enterococcus faecalis* (8.0%, n = 4). Table 2 summarizes pathogen species distribution, primary clinical ward sources, and species-specific AMR prevalence rates.

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Table 2 Bacterial Etiology & Species-Specific AMR Rates

Bacterial Species	Gram Stain	Isolates n (%)	Primary Ward Source	AMR Positive n (%)
Escherichia coli	Gram (-)	18 (36.0%)	Internal Med, OB/GYN	12 (66.7%)
Staphylococcus aureus	Gram (+)	13 (26.0%)	Surgery, Pediatrics	7 (53.8%)
Klebsiella pneumoniae	Gram (-)	20.0% (10)	Internal Med, Surgery	7 (70.0%)
Pseudomonas aeruginosa	Gram (-)	5 (10.0%)	Surgery	3 (60.0%)
Enterococcus faecalis	Gram (+)	4 (8.0%)	Internal Med	1 (25.0%)
Total Bacterial Cohort	--	**50 (100.0%)**	--	**30 (60.0%)**

Species-specific analysis revealed that *Klebsiella pneumoniae* exhibited the highest proportion of resistant isolates (70.0%, n = 7/10), followed closely by Escherichia coli (66.7%, n = 12/18) and Pseudomonas aeruginosa (60.0%, n = 3/5). Among Gram-positive isolates, 53.8% (n = 7/13) of Staphylococcus aureus isolates were non-susceptible to standard beta-lactam therapy.

- *Comprehensive Antimicrobial Susceptibility Testing (AST) Profiles*

Susceptibility testing across ten standard antibacterial agents demonstrated wide variations in non-susceptibility rates. High levels of resistance were recorded for broad-spectrum agents commonly used for empiric therapy in district facilities. Table 3 presents the detailed susceptibility and non-susceptibility profiles across all tested antimicrobial agents.

Table 3 Comprehensive Antibiotic Susceptibility & Resistance Profiles (N)

Antimicrobial Agent	Class	Tested Isolates	Susceptible n (%)	Intermediate n (%)
Ampicillin	Penicillin	36	4 (11.1%)	2 (5.6%)
Trimethoprim-Sulfamethoxazole	Folate Pathway	50	10 (20.0%)	3 (6.0%)
Ceftriaxone	3rd Gen Cephalosporin	42	15 (35.7%)	4 (9.5%)
Amoxicillin-Clavulanate	Beta-lactam/Inhibitor	45	18 (40.0%)	5 (11.1%)
Ciprofloxacin	Fluoroquinolone	50	23 (46.0%)	4 (8.0%)
Erythromycin	Macrolide	17	8 (47.1%)	2 (11.8%)
Cefotaxime	3rd Gen Cephalosporin	38	19 (50.0%)	3 (7.9%)
Gentamicin	Aminoglycoside	50	37 (74.0%)	3 (6.0%)
Vancomycin	Glycopeptide	17	14 (82.4%)	1 (5.9%)
Imipenem	Carbapenem	50	46 (92.0%)	1 (2.0%)

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26nov601

➢ *Multidrug Resistance (MDR) Patterns*

Multidrug resistance (MDR), defined as non-susceptibility to at least one agent in three or more distinct antimicrobial classes (Magiorakos et al., 2012), was evaluated across all 50 isolates. Overall, 44.0% (n = 22/50) of clinical isolates met the criteria for MDR status. Figure 1 illustrates the distribution of isolates by the number ofnon-susceptible drug classes.

Table 4 Multi-Class Resistance Profiles Across Isolated Pathogens

Resistance Category	Definition / Class Count	Isolates n (%)	Dominant Pathogens
Fully Susceptible	Resistant to 0 classes	20 (40.0%)	*E. coli* (6), *S. aureus* (6)
Single-Class Resistance	Resistant to 1 class	4 (8.0%)	*E. coli* (2), *E. faecalis* (2)
Dual-Class Resistance	Resistant to 2 classes	4 (8.0%)	*S. aureus* (2), *P. aeruginosa* (2)
MDR Category 1	Resistant to 3 classes	11 (22.0%)	*E. coli* (5), *K. pneumoniae* (4)
MDR Category 2	Resistant to 4 classes	7 (14.0%)	*K. pneumoniae* (3), *S. aureus* (3)
Extensive MDR	Resistant to ≥ 5 classes	4 (8.0%)	*E. coli* (2), *P. aeruginosa* (1)
Total Cohort	--	**50 (100.0%)**	--

Among the 22 MDR isolates, Gram-negative bacilli accounted for 72.7% (n = 16/22), with *Klebsiella pneumoniae* exhibiting the highest proportion of MDR strains relative to its species total (70.0%, n = 7/10). Among *Staphylococcus aureus* isolates, 30.8% (n= 4/13) met the clinical definition for methicillin-resistant *S. aureus* (MRSA) phenotype based on oxacillin/cefoxitin screening resistance.

IV. DISCUSSIONS

This study established a baseline laboratory-confirmed AMR prevalence rate of 60.0% among tested inpatient isolates at Kirehe District Hospital, Rwanda. This finding underscores the significant magnitude of bacterial antimicrobial resistance in secondary-level rural district healthcare facilities. The observed 60.0% prevalence rate aligns closely with recent surveillance reports across subSaharan Africa. The WHO Global Antibiotic Resistance Surveillance Report documented widespread resistance exceeding 50% for common Gram-negative pathogens across developing health systems (WHO, 2025b). Similarly, a landmark meta-analysis of healthcareassociated infections in 20 African nations revealed endemic resistance rates ranging between 52% and 68% among hospitalized inpatient cohorts (Bunduki et al., 2024).

➢ *Pathogen Etiology & Specimen Yield*

Gram-negative Enterobacterales dominated the microbiological profile at Kirehe District Hospital, representing 56.0% of total isolates, led by *Escherichia coli* (36.0%) and *Klebsiella pneumoniae* (20.0%). This species breakdown reflects global patterns in hospitalacquired and severe community-onset bacterial infections (Murray et al., 2022). Urine (36.0%) and wound swabs/pus (34.0%) constituted the primary specimen sources, mirroring clinical admission patterns in Internal Medicine and General Surgery wards.

The high isolation rate of *E. coli* in urine specimens (66.7% AMR rate) highlights significant resistance challenges in managing complicated urinary tract infections (UTIs). Furthermore, *K. pneumoniae* demonstrated the highest overall species resistance rate (70.0%), consistent with findings from referral hospital surveillance at CHUK in Kigali, which identified *Klebsiella* species as major drivers of extended-spectrum beta-lactamase (ESBL) production (Munyemana et al., 2025).

➢ *Analysis ofAntibiotic Non-Susceptibility Profiles*

The extremely high non-susceptibility rates observed against ampicillin (88.9%) and trimethoprim-sulfamethoxazole (80.0%) indicate that these traditional first-line agents have lost clinical efficacy for empiric treatment of bacterial infections at Kirehe District Hospital. High baseline plasmid-mediated resistance to cotrimoxazole is widely documented across East Africa, driven by decades of widespread unmonitored use and prophylaxis protocols (Katakweba et al., 2023).

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26nov601

Of significant clinical concern is the 64.3% non-susceptibility rate to ceftriaxone, a third-generation cephalosporin that serves as the cornerstone empiric parenteral treatment for severe inpatient infections in Rwandan district hospitals (Ministry of Health Rwanda, 2023). Resistance to third-generation cephalosporins in Enterobacterales strongly suggests the presence of ESBL enzymes or AmpC beta-lactamases, which severely restrict oral step-down options (Laxminarayan et al., 2023).

Conversely, aminoglycosides (gentamicin, 74.0% susceptible) and carbapenems (imipenem, 92.0% susceptible) demonstrated preserved in-vitro antimicrobial activity. Carbapenem resistance remained low (8.0%), confirming that carbapenems remain vital reserve agents for life-threatening multidrug-resistant Gram-negative infections in secondary care facilities.

- *High Burden of Multidrug Resistance (MDR)*

The 44.0% MDR prevalence documented in this cohort is alarming. MDR bacterial strains severely limit therapeutic choices, force reliance on costlier second- and third-line reserve medications, prolong inpatient hospital stay, and elevate clinical treatment failure rates (Bunduki et al., 2024). The high MDR prevalence among Gram-negative isolates underscores an urgent need for routine microbiology diagnostics prior to initiating broad-spectrum antimicrobial therapy.

- *Methodological Limitations*

This study has limitations: (1) The small sample size (N = 50) derived from selective diagnostic testing limits generalizability to all outpatient community settings in Kirehe District; (2) Molecular characterization of resistance genes (e.g., PCR detection of *bla*CTX_M, *bla*NDM, or *mecA*) was unavailable due to local laboratory resource constraints; and (3) Clinical outcome tracking (mortality vs cure) was beyond the scope ofthis single-objective prevalence paper.

V. CONCLUSION

This baseline microbiological study demonstrates a high prevalence of antimicrobial resistance (60.0%) and multidrug resistance (44.0%) among tested hospitalized patients at Kirehe District Hospital, Rwanda. Gram-negative Enterobacterales (*E. coli* and *K. pneumoniae*) were the predominant resistant pathogens. High nonsusceptibility rates to standard empiric antibacterials—particularly ampicillin, co-trimoxazole, and ceftriaxone—indicate that conventional empiric regimens are increasingly compromised. Routine laboratory culture, AST surveillance, and carbapenem preservation are vital to optimize patient care in rural district health systems.

RECOMMENDATIONS

Based on the epidemiological and AST findings of this study, the following specific recommendations are formulated for clinical practice and facility administration:

- *Establish Cumulative Hospital Antibiograms:*

The Kirehe District Hospital Microbiology Laboratory should publish bi-annual cumulative antibiograms summarizing pathogen isolation rates and drug susceptibility trends to guide clinical empiric antibiotic selection.

- *Revise Empirical Treatment Protocols:*

Hospital clinical leadership should re-evaluate local empirical antibiotic guidelines, restricting ampicillin and co-trimoxazole for systemic inpatient infections, and exercising judicious stewardship over ceftriaxone prescribing.

- *Strengthen Diagnostic Microbiology Utilization:*

Clinicians should be encouraged to routinely request blood, urine, and wound cultures prior to initiating antimicrobial therapy, particularly for nonresponsive or severe inpatient admissions.

- *Preserve Reserve Antibiotic Classes:*

Establish strict access authorization controls for carbapenems (imipenem, meropenem) and vancomycin to prevent the emergence of carbapenem-resistant Enterobacterales (CRE) within the district facility.

- *Infection Prevention and Control (IPC):*

Intensify hand hygiene compliance, surface disinfection, and contact precautions for patients colonized or infected with MDR isolates to prevent nosocomial crosstransmission.

- *Future Research:*

Expand molecular surveillance to identify specific resistance genes (ESBL, carbapenemases) and evaluate the clinical outcomes associated with MDR bacterial infections in district hospitals across Rwanda.

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