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Temporal Trends and Antimicrobial Susceptibility Patterns of ESKAPE Pathogens Recovered from Clinical Specimens in Western Oromia, Ethiopia: Implications for AMR Surveillance and Antimicrobial Stewardship

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Abstract

Background: ESKAPE pathogens are the leading causes of healthcare-associated and community-acquired infections worldwide. Despite this, there is limited evidence on temporal trends and antimicrobial susceptibility patterns of these pathogens in Western Oromia, Ethiopia. This study assessed temporal trends and antimicrobial susceptibility patterns of ESKAPE pathogens recovered from clinical specimens.

Methods: Microbiology records from 2023–2025 were analyzed to characterize the antimicrobial susceptibility patterns and temporal trends of ESKAPE pathogens. The pathogens were identified using standard microbiological methods, and AST was performed using the Kirby–Bauer disk diffusion method. Data were entered into EpiData version 4.6 and analyzed using SPSS version 27. Descriptive statistics, chi-square tests, and the Cochran–Armitage trend test were used to assess distributions and temporal changes in ESKAPE pathogens. Statistical significance was determined at $p < 0.05$.

Results: Of the total 954 bacteria isolated, ESKAPE pathogens accounted for 568 (59.5%) with *S. aureus* (354; 62.3%) and *K. pneumoniae* (94; 16.5%) being predominant. ESKAPE prevalence increased from 56.5% in 2023 to 65.0% in 2024, then declined to 57.1% in 2025. The distribution of ESKAPE and non-ESKAPE pathogens did not differ significantly across the three study years (p=0.056). Antimicrobial susceptibility testing revealed that *S. aureus* isolates showed an alarming resistance of 95 – 99.0% to penicillin over the three years. *K. pneumoniae* indicated 79.4% resistance to third-generation cephalosporins (ceftriaxone and ceftazidime) in 2025. In addition, high resistance of 76.5% was observed against cefepime, a fourth-generation cephalosporin. Moreover, *Pseudomonas* isolates demonstrated increased resistance rates to meropenem from 2023 through 2025 accounting for 50.0%, 57.1%, and 71.1%, respectively. Among the ESKAPE isolates, 351 (61.8%) were multidrug-resistant (MDR), predominantly *S. aureus* (243; 69.2%) and *K. pneumoniae* (71; 20.2%).

Conclusion: The consistently high and variable occurrence of ESKAPE pathogens, rising antimicrobial resistance, and the burden of MDR organisms highlight the pressing necessity to enhance antimicrobial stewardship and infection prevention and control (IPC) strategies. Continuous AMR monitoring, data-driven antibiotic utilization, and successful IPC measures are crucial to reduce the rise and spread of resistant ESKAPE organisms.

Keywords: ESKAPE pathogens, antimicrobial resistance, multidrug resistance, multiple antibiotic resistance index, antimicrobial stewardship

Introduction

Antimicrobial resistance (AMR) has emerged as one of the most critical public health challenges of the 21st century, consistently eroding years of medical advancements and jeopardizing the core of contemporary healthcare (1). It has emerged as one of the most significant global public health threats, accounting for an estimated 1.3 million deaths worldwide in 2019 and projected to contribute to approximately 10 million deaths annually by 2050 (2). The increasing emergence and dissemination of resistant bacterial pathogens have led to prolonged hospitalization, treatment failure, increased healthcare expenditures, and excess mortality worldwide (3). The burden of AMR is particularly substantial in low- and middle-income countries, where limited diagnostic capacity, inadequate infection prevention and control measures, and inappropriate antimicrobial use facilitate the spread of resistant microorganisms (4,5).

Among the pathogens contributing most significantly to the global AMR crisis are the ESKAPE pathogens. The ESKAPE pathogens are a particular elite group of multidrug-resistant (MDR) organisms that disproportionately contribute to the global burden of AMR (6). They account for the vast majority of severe healthcare-associated infections (HAIs) and exhibit extraordinary capacities to evade conventional antimicrobial regimens (7,8). Consequently, the pathogens are included in the World Health Organization (WHO) bacterial priority pathogen list due to high antimicrobial resistance, limited treatment options, and the need for ongoing surveillance and new antimicrobial strategies (9).

The intricate interaction of innate and acquired resistance mechanisms accounts for the pathogenicity and clinical persistence of ESKAPE infections (10). Gram-positive members (*E. faecium* and *S. aureus*) commonly resist key cell-wall agents through *vanA/vanB*-mediated VRE and *mecA*-encoded PBP2a in MRSA (11,12). In addition, Gram-negative ESKAPE pathogens (*K. pneumoniae, A. baumannii, P. aeruginosa, and Enterobacter* spp.) present significant clinical challenges by means of ESBLs, AmpC, and carbapenemases such as KPC, NDM, and OXA-48 (13,14). These bacteria manifest MDR, extensively drug-resistant (XDR), and pandrug-resistant (PDR) phenotypes that severely restrict first-line and last-resort therapeutic choices such as carbapenems, polymyxins, and novel beta-lactam/beta-lactamase inhibitor combinations via decreased outer membrane permeability, overexpressed efflux pump systems, and strong biofilm-forming capabilities (15–17).

Single-center cross-sectional studies offer a glimpse of local resistance patterns but do not reflect the ongoing changes in bacterial evolution due to continuous antimicrobial pressure (18). Longitudinal monitoring of temporal trends across various clinical anatomical locations, including blood, the lower respiratory tract, the urinary tract, and skin/soft tissue samples, is

essential (19). Grasping the changes in resistance patterns among ESKAPE pathogens is essential for making clinical decisions and managing hospital infections. The dynamics of resistance vary notably over time, influenced by changes in antimicrobial usage, selective pressures specific to different wards, guidelines for empirical therapy, and the clonal growth of MDR or XDR lineage clones (20,21).

Despite their risk, multi-year ESKAPE monitoring that incorporates specimen, temporal, co-resistance, and ward-level trends is still restricted. Long-term monitoring is crucial for identifying shifts in resistance patterns, evaluating the effects of stewardship and infection control, and informing tailored antimicrobial policies. Evaluating routine microbiology data can illustrate evolving ESKAPE epidemiology and resistance trends. The present study aimed to assess the temporal trends, distribution, and antimicrobial resistance characteristics of ESKAPE pathogens recovered from clinical specimens over a three-year period. By describing changes in pathogen occurrence and antimicrobial susceptibility over time, this study seeks to generate evidence that can support empirical antimicrobial selection, antimicrobial stewardship, infection prevention and control, and strengthening of local AMR surveillance.

Methods and materials

Study setting, design and period

The study was conducted at the Nekemte Public Health Research and Referral Laboratory Center, located in Nekemte Town, western Ethiopia. The center serves as a regional referral laboratory and provides comprehensive diagnostic services for patients referred from health facilities within its catchment area. In addition to routine laboratory diagnostics, the center conducts public health and biomedical research aimed at generating evidence to support disease prevention and control activities. The laboratory center also plays a key role in capacity building, strengthen laboratory quality management systems, improve diagnostic services, and enhance the implementation of national health programs. Furthermore, the Nekemte Public Health Research and Referral Laboratory Center is currently one of the AMR surveillance sentinel sites in Ethiopia. As part of the national AMR surveillance network, the center performs culture and antimicrobial susceptibility testing of priority bacterial pathogens and contributes surveillance data to support national efforts in monitoring AMR trends, guiding treatment recommendations, and informing public health interventions. A retrospective cross-sectional study was conducted. Cultures results of three years (2023-2025) were extracted and analyzed.

Population

The source population comprised all bacterial isolates recovered from clinical specimens submitted to the microbiology laboratory of Nekemte Public Health Research and Referral Laboratory Center during the study period. The study population consisted of all laboratory-confirmed ESKAPE pathogens, isolated from clinical specimens between January 2023 and December 2025.

Eligibility Criteria

Inclusion Criteria

All non-duplicate clinical isolates of ESKAPE pathogens recovered from routine clinical specimens (e.g., blood, urine, respiratory secretions, pus/wound swabs, sterile body fluids) during the study period were included.

Exclusion Criteria

Records were excluded if they contained incomplete demographic, microbiological, or antimicrobial susceptibility data. Duplicate isolates obtained from the same patient during the same infection episode were also excluded.

Variables

The primary outcome variables of this study were prevalence of ESKAPE bacteria and antimicrobial susceptibility profiles over the three years. MDR was determined based on the susceptibility patterns of the isolates to the tested antimicrobial agents. Resistance to individual antibiotics and the annual prevalence of ESKAPE bacteria were also assessed to evaluate temporal trends in antimicrobial resistance over the study period. Multiple antibiotic resistance index (MARI) was determined by focusing on number of antibiotics tested and number of antibiotics to which the organism was resistant.

The independent variables included patient demographic characteristics, such as age and sex, as well as the year of specimen collection. Moreover, additional variables such as patient care setting (inpatient or outpatient), ward or clinic of specimen origin, and referring health facility were considered. These variables were used to describe the distribution of ESKAPE isolates and to assess variations in antimicrobial resistance patterns across different population groups and time periods.

Sample Size Determination and Sampling Technique

A census sampling method encompassed all eligible ESKAPE isolates identified throughout the study duration. No calculation of sample size was conducted. Microbiology records, results of antimicrobial susceptibility, and patient information were examined, omitting incomplete or duplicate entries and contaminated cultures. All eligible isolates were incorporated in the final analysis.

Data Collection Tool and Procedure

Data were collected using a structured checklist developed to capture demographic, specimen, bacterial identification, and antimicrobial susceptibility data. The checklist was reviewed for completeness and consistency with the study objectives before data collection. Trained laboratory professionals retrospectively extracted data from microbiology culture and AST records for the three-year study period. Data quality was ensured through daily checks for completeness, accuracy, and consistency, while incomplete, duplicate, and contaminated records were excluded. The cleaned data were coded, entered into a EpiData and prepared for statistical analysis in SPSS.

Operational Definitions

Multidrug resistance: Non-susceptibility to at least one agent in three or more antimicrobial categories (22).

Multiple antibiotic resistance index (MARI): MARI was calculated by dividing the number of antibiotics to which the isolate was resistant to the total number of antibiotics tested against it (23).

Data Quality Assurance

Data quality was ensured using a standardized checklist and trained laboratory professionals. Extracted data were reviewed for completeness, accuracy, and consistency, with discrepancies verified against original records. Incomplete records and duplicate isolates from the same infection episode were excluded according to predefined criteria.

All microbiological procedures followed standard operating procedures. Internal quality control used recommended ATCC strains, including *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 25923. The laboratory also participates in national AMR surveillance and external quality assessment programs. Data were cleaned, coded, and validated before analysis.

Data Processing and Analysis

Data were checked, cleaned, coded, and entered into EpiData 4.6, then exported to SPSS 27 for analysis. Descriptive statistics summarized demographic characteristics, specimen distribution, ESKAPE pathogens, and antimicrobial susceptibility patterns. ESKAPE prevalence and antimicrobial resistance rates were calculated as proportions of bacterial isolates and isolates tested, respectively. MDR was defined as non-susceptibility to at least one agent in three or more antimicrobial classes.

Temporal trends in the prevalence of ESKAPE pathogens, MDR rates, MARI and resistance to commonly tested antimicrobial agents were assessed across the five-year study period. Annual proportions were calculated and presented using tables and graphs to illustrate changes in pathogen distribution and antimicrobial resistance patterns over time. Associations between categorical variables were evaluated using the chi-square test. A p-value of <0.05 was considered statistically significant. The findings were presented using tables, figures, and narrative descriptions.

Results

General Characteristics of Study Participants

During the three-year period, a total of 1967 patients were tested among which 935 (47.5%) and the remaining 1032 (52.5%) were females. The mean age of the patients was 27.4 ± 19.1 years. Facility-based analysis revealed that majority of the study participants were referred from hospitals, accounting for 1649 (83.8%) (Table 1).

Table 1: General characteristics of study participants (N = 1967)

Variable		Frequency	Percentage
Age (years)	<5	325	16.5
	5-14	182	9.3
	15-24	353	17.9
	25-34	480	24.4
	≥35	627	21.9
Sex	Male	935	47.5
	Female	1032	52.5
Referring health facility	Hospitals	1649	83.8
	Health centers	86	4.4
	Clinics	232	11.8
Location	Outpatient department (OPD)	1504	76.5
	Inpatient department (IPD)	463	23.5
Clinical specimens	Ear discharge	589	29.9
	Blood	331	16.8
	Urine	522	26.5
	Pus	156	7.9
	Genital specimens	189	9.6
	Sputum	83	4.2
	Others*	97	4.9

***Nasal swab, throat swab, CSF, body fluids, stool**

Culture Results and Isolated Bacterial Pathogens

Over the three years, 1967 clinical specimens were cultured among which 950 (48.3%) yielded positive results. Moreover, a total of 954 bacterial isolates were identified. Of the total isolates, ESKAPE bacteria accounted for 568 (59.5%) (Figure 1).

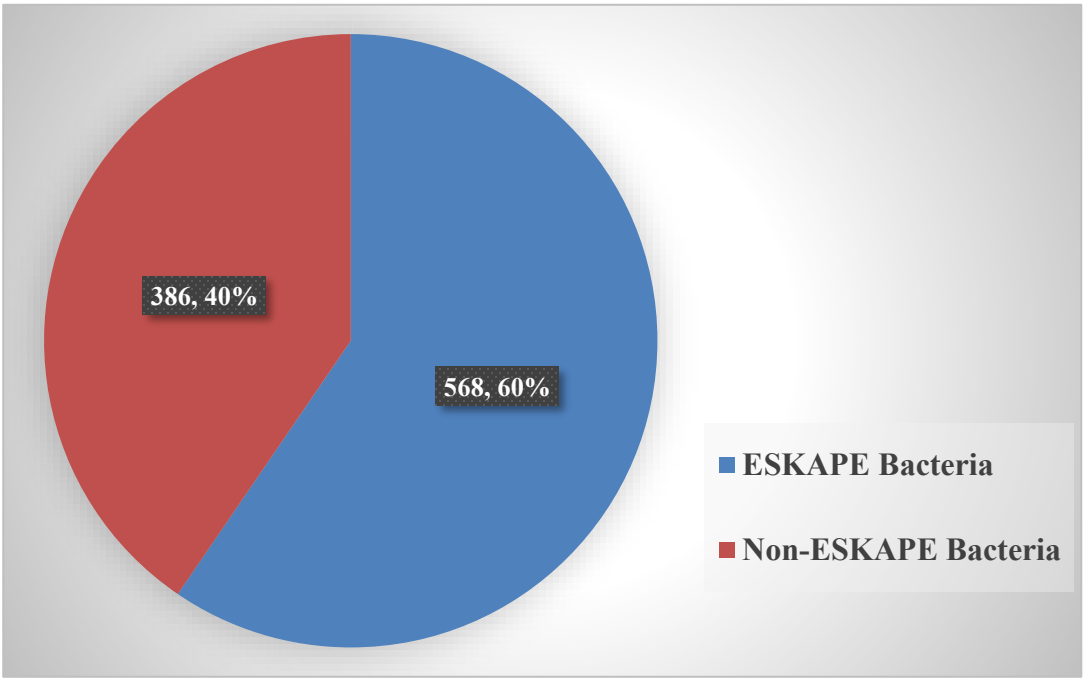


Figure 1: Bacterial isolation over the three years at Nekemte Public Health Research and Referral Laboratory Center, Nekemte, Ethiopia, 2023-2025

Distribution of ESKAPE Pathogens among Patient Characteristics

According to this study, the highest number of ESKAPE isolates were recovered from patients aged ≥35 years followed by under 5 children accounting for 154 (27.1%) and 141 (24.8%), respectively. However, the distribution of ESKAPE and non-ESKAPE pathogens did not differ significantly across age groups ($\chi^2 = 8.235$, p=0.083). Of the total hospital isolates, 500 (61.1%) were found to be ESKAPE pathogens. It was found that the distribution of the bacterial isolates had shown significant difference across health facilities (χ^2=7.031, p=0.030) (Table 2).

Table 2: Distribution of ESKAPE pathogens among patient characteristics recovered from clinical specimens at Nekemte Public Health Research and Referral Laboratory Center, Nekemte, Ethiopia, 2023-2025

Variable	Isolated bacteria			χ^2	*p-value*
	ESKAPE, N (%)	Non-ESKAPE, N (%)	Total, N (%)		
Age (years)					
<5	141 (62.4)	85 (37.6)	226 (23.7)	8.235	0.083
5-14	83 (68.0)	39 (32.0)	122 (12.8)		
15-24	88 (55.0)	72 (45.0)	160 (16.8)		
25-34	102 (54.0)	87 (46.0)	189 (19.8)		
≥35	154 (60.0)	103 (40.0)	257 (26.9)		
Sex					
Male	286 (58.1)	206 (41.9)	492 (51.6)	0.837	0.360
Female	282 (61.0)	180 (39.0)	462 (48.4)		
Referring health facility type					
Hospital	500 (61.1)	319 (38.9)	819 (85.8)	7.031	**0.030**
Health center	15 (41.7)	21 (58.3)	36 (3.8)		
Clinic	53 (53.5)	46 (46.5)	99 (10.4)		
Patient location					
OPD	448 (57.3)	334 (42.7)	782 (82.0)	9.113	**0.003**
IPD	120 (69.8)	52 (30.2)	172 (18.0)		
Clinical specimens					
Ear discharge	341 (60.4)	224 (39.6)	565 (59.2)	57.830	**<0.001**
Blood	72 (77.4)	21 (22.6)	93 (9.7)		
Urine	50 (50.5)	49 (49.5)	99 (10.4)		
Pus	80 (64.0)	45 (36.0)	125 (13.1)		
Sputum	14 (63.6)	8 (36.4)	22 (2.3)		
Others*	11 (22.0)	39 (78.0)	50 (5.2)		

***Body fluids, nasal swab **Significant at p<0.05**

Prevalence of ESKAPE Pathogens

In this study, Gram-positive ESKAPE pathogens constituted 356 (62.7%) of all isolates, whereas Gram-negative ESKAPE pathogens accounted for 212 (37.3%). Of the total ESKAPE bacteria recovered over the three years, the predominant isolates were *S. aureus* 354 (62.3%) followed by *K. pneumoniae* 94 (16.5%). On the other hand, *Enterobacter* species and *Enterococcus* species were found to be rare bacteria reported by this study, accounting for 9 (1.6%) and 2 (0.4%), respectively (Table 3).

Table 3: Prevalence of ESKAPE pathogens recovered from clinical specimens at Nekemte Public Health Research and Referral Laboratory Center, Nekemte, Ethiopia, 2023-2025

ESKAPE Pathogen	Frequency	Percentage (%)
Gram-positive		
S. aureus	354	62.3
Enterococcus species	2	0.4
Total	**356**	**62.7**
Gram-negative		
K. pneumoniae	94	16.5
Pseudomonas species	82	14.4
Acinetobacter species	27	4.8
Enterobacter species	9	1.6
Total	**212**	**37.3**

Distribution of ESKAPE Pathogens with Respect to Patient Characteristics

Age-wise analysis revealed that *S. aureus* and *K. pneumoniae* were predominantly isolated from under five children, accounting for 95 (26.8%) and 28 (29.8%) respectively. Moreover, majority of *Pseudomonas* species 25 (30.5%) were recovered from patients aged at least 35 years. From the total 354 *S. aureus* isolates, 308 (87.0%) were recovered from hospitals. In addition, majority 288 (81.4%) were from outpatient departments. Moreover, about 230 (65.0%) of *S. aureus* isolates were recovered from ear discharge followed by pus/wound 54 (15.3%). Similarly, 86 (91.5%) and 69 (73.4%) of the *K. pneumoniae* isolates were recovered from hospitals OPDs, with ear discharge being the predominant specimen source (41, 43.6%), followed by urine (21, 22.3%) and blood (18, 19.1%). Furthermore, *Pseudomonas* species was predominantly isolated from hospital settings (75, 91.5%) with 63 (76.8%) of the isolates being from OPDs. It was also found that the primary source of clinical specimen for this bacterium was ear discharge (54, 65.9%) (Table 4).

Table 4: Distribution of each ESKAPE pathogen recovered from clinical specimen at Nekemte Public Health Research and Referral Laboratory Center, Nekemte, Ethiopia, 2023-2025

Characteristics	ESKAPE pathogens					
	***Enterococcus* spp.**	***S. aureus***	***K. pneumoniae***	***Acinetobacter* spp.**	***Pseudomonas* spp.**	***Enterobacter* spp.**
Age (year)						
<5	1 (50.0)	95 (26.8)	28 (29.8)	7 (25.9)	8 (9.8)	3 (33.3)
5-14	0 (0.0)	55 (15.5)	9 (9.6)	5 (18.5)	14 (17.1)	0 (0.0)
15-24	0 (0.0)	50 (14.1)	15 (16.0)	4 (14.8)	17 (20.7)	1 (11.1)
25-34	1 (50.0)	65 (18.4)	16 (17.0)	2 (7.4)	18 (22.0)	3 (33.3)
≥35	0 (0.0)	89 (25.1)	26 (27.7)	9 (33.3)	25 (30.5)	2 (22.2)
Sex						
Male	2 (100)	187 (52.8)	41 (43.6)	13 (48.1)	40 (48.4)	2 (22.2)
Female	0 (0.0)	167 (47.2)	53 (56.4)	14 (51.9)	42 (51.2)	7 (77.8)
Referring health facility						
Hospitals	2 (100)	308 (87.0)	86 (91.5)	25 (92.6)	75 (91.5)	7 (77.8)
Health centers	0 (0.0)	13 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Clinics	0 (0.0)	33 (9.3)	8 (8.5)	2 (7.4)	7 (8.5)	2 (22.2)
Patient location						
OPD	1 (50.0)	288 (81.4)	69 (73.4)	23 (85.2)	63 (76.8)	7 (77.8)
IPD	1 (50.0)	66 (18.6)	25 (26.6)	4 (14.8)	19 (23.2)	2 (22.2)
Clinical specimens						
Ear discharge	1 (50.0)	230 (65.0)	41 (43.6)	19 (70.4)	54 (65.9)	4 (44.4)
Blood	0 (0.0)	38 (10.7)	18 (19.1)	3 (11.1)	8 (9.8)	1 (11.1)
Urine	0 (0.0)	19 (5.4)	21 (22.3)	3 (11.1)	6 (7.3)	2 (22.2)
Pus	1 (50.0)	54 (15.3)	8 (8.5)	0 (0.0)	11 (13.4)	1 (11.1)
Sputum	0 (0.0)	6 (1.7)	4 (4.3)	2 (7.4)	2 (2.4)	0 (0.0)
CSF	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)
Others*	0 (0.0)	7 (2.0)	1 (1.1)	0 (0.0)	1 (1.2)	1 (11.1)

CSF – Cerebrospinal fluid ***Body fluids (peritoneal fluid, synovial fluid, pleural fluid), Stool**

Temporal Trends of ESKAPE Pathogens

During the three-year study period, 954 bacterial isolates were recovered, of which 568 (59.6%) were ESKAPE pathogens and 386 (40.4%) were non-ESKAPE pathogens. The proportion of ESKAPE pathogens varied over time, accounting for 56.5% (139/246) of isolates in 2023, increasing to 65.0% (204/314) in 2024, and subsequently declining to 57.1% (225/394) in 2025. Although the absolute number of ESKAPE isolates increased each year (139, 204, and 225 isolates in 2023, 2024, and 2025, respectively), the annual proportion peaked in 2024 before decreasing in 2025. The distribution of ESKAPE and non-ESKAPE pathogens did not differ significantly across the three study years ($\chi^2 = 5.75$, $p = 0.056$) (Table 5). Although the absolute number of ESKAPE isolates increased annually, the Cochran–Armitage test showed no significant linear trend in the proportion of ESKAPE pathogens over time (χ^2trend = 0.030, $p = 0.862$) (Figure 2).

Table 5: Annual distribution of ESKAPE and non-ESKAPE pathogens recovered from clinical specimens at Nekemte Public Health Research and Referral Laboratory Center, Nekemte, Ethiopia, 2023-2025

Bacterial isolates		**2023**	**2024**	**2025**	**Total**
ESKAPE, N (%)		139	204	225	568
Non-ESKAPE, N (%)		107	110	169	386
Total isolate, N (%)		246	314	394	954
ESKAPE prevalence, %		56.5	65.0	57.1	59.5
χ^2		5.750			
P-value		0.056			
Cochran-Armitage test	χ^2trend	0.030			
	p-value	0.862			

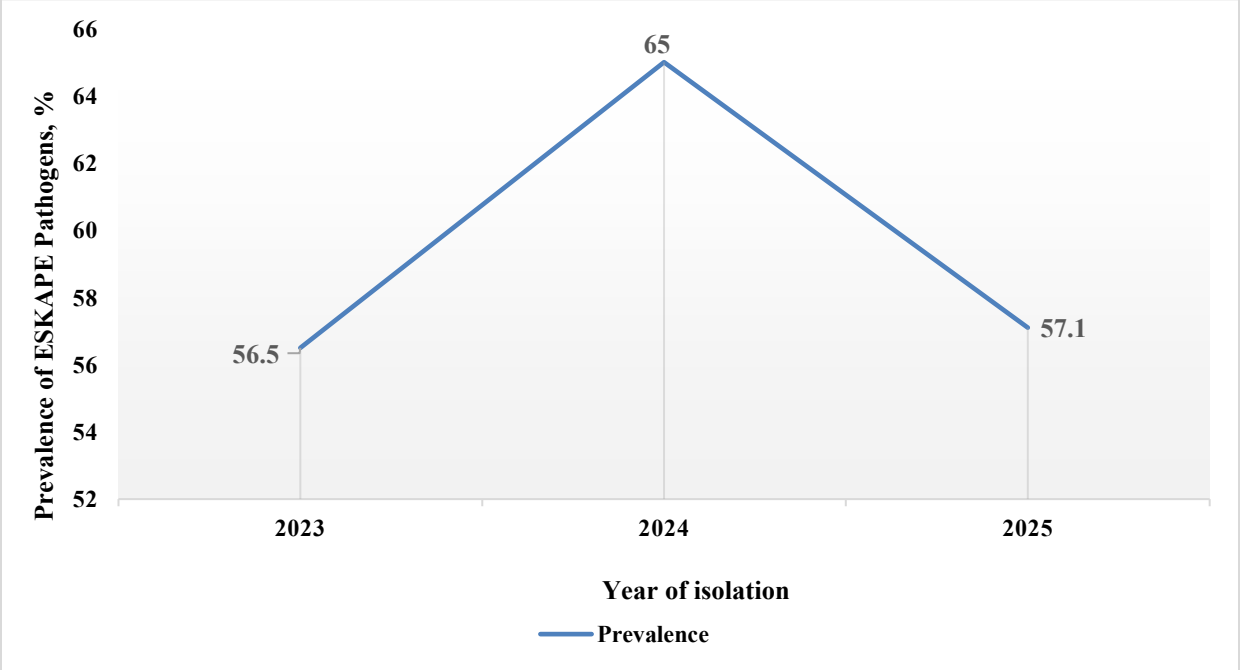


Figure 2: Trends of ESKAPE pathogens at Nekemte Public Health Research and Referral Laboratory Center, Nekemte, Ethiopia, 2023-2025

Trends of Antibiotic Resistance among ESKAPE Pathogens

Among *S. aureus* isolates, penicillin resistance remained consistently high during the study period, although a slight decline was observed from 99.0% in 2023 to 95.6% in 2024 and 95.0% in 2025. Conversely, susceptibility increased from 1.0% in 2023 to 4.4% in 2024 and 5.0% in 2025. Over the three years, gentamicin was found to be effective antibiotic to which only 15.7%, 8.8% and 20.9% were resistant in 2023, 2024 and 2025, respectively. The highest resistance to tetracycline was observed in 2025 when 65.5% were resistant. In 2023, half of the S. aureus isolates demonstrated resistance to ciprofloxacin, slightly decreasing to 38.8% in 2025. Similarly, resistance to azithromycin decreased from 55.9% in 2023 to 35.3% in 2025 (Figure 3). Furthermore, the prevalence of MRSA showed fluctuating trends over the three years with alarming rates (Figure 4).

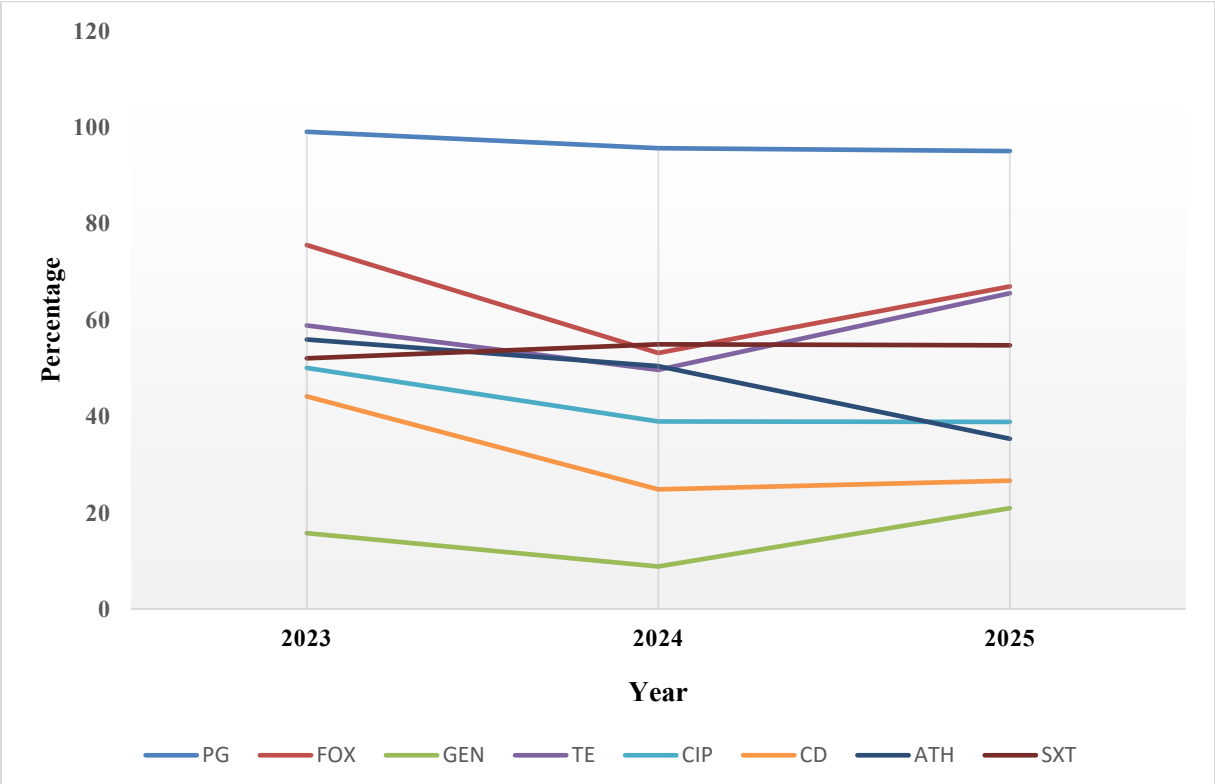


Figure 3: Antibiotic resistance trends of *S. aureus* (2023-2025)

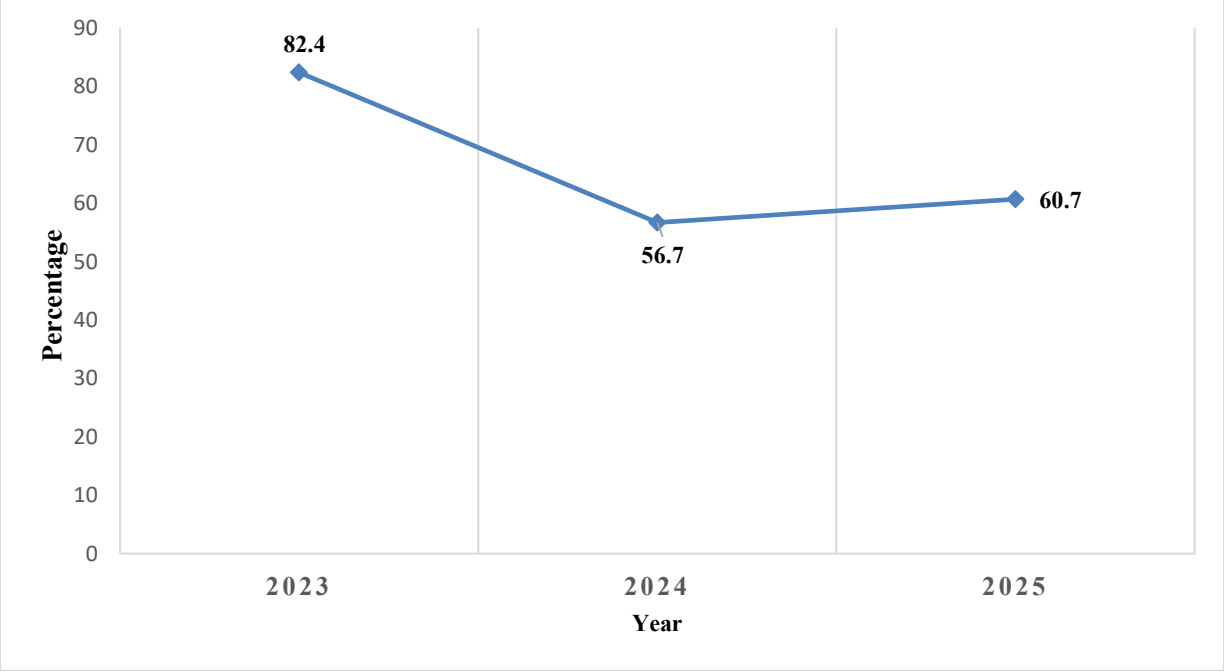


Figure 4: Trends of methicillin-resistant *S. aureus* (2023-2025)

As shown in figure 5, two beta-lactam combination antibiotics were tested over the three-year period. The resistance of *K. pneumoniae* to amoxicillin–clavulanic acid showed an increasing trend over the three-year study period. Resistance increased from 61.1% in 2023 to 61.9% in 2024, followed by a marked increase to 73.5% in 2025. The findings indicate a substantial and worsening resistance burden to AMC among *K. pneumoniae* isolates, particularly in 2025. Piperacillin-tazobactam was another combination agent to which 38.9% of the isolates were resistant in 2023. However, in 2024, the resistance rate was declined to 19.0% but sharply increased to 61.8% in 2025. Of the carbapenems, meropenem resistance was 38.9% in 2023, decreasing to 16.7% but raising to 23.5% in 2025. Of the two aminoglycosides tested, the highest resistance was observed with gentamicin 41.2% in 2025 but the observed resistance to amikacin was almost similar over the three years. It was also revealed that 73.5% and 79.4% of *K. pneumoniae* isolates were resistant to tetracycline and trimethoprim-sulfamethoxazole, respectively. Alarming resistance was observed with two third generation cephalosporins. Resistance to ceftriaxone was seen among 66.7% of the isolates in 2023, slightly decreasing to 64.3% with rapid increase to 79.4% in 2025. In addition, 61.1% of them were resistant in 2023, coming down to 57.1% but raising to 79.4% in 2025. Resistance to a fourth-generation cephalosporin, cefepime, showed a fluctuating trend from 44.4% in 2023, 38.1% in 2024 but dramatically raising to 76.5% in 2025.

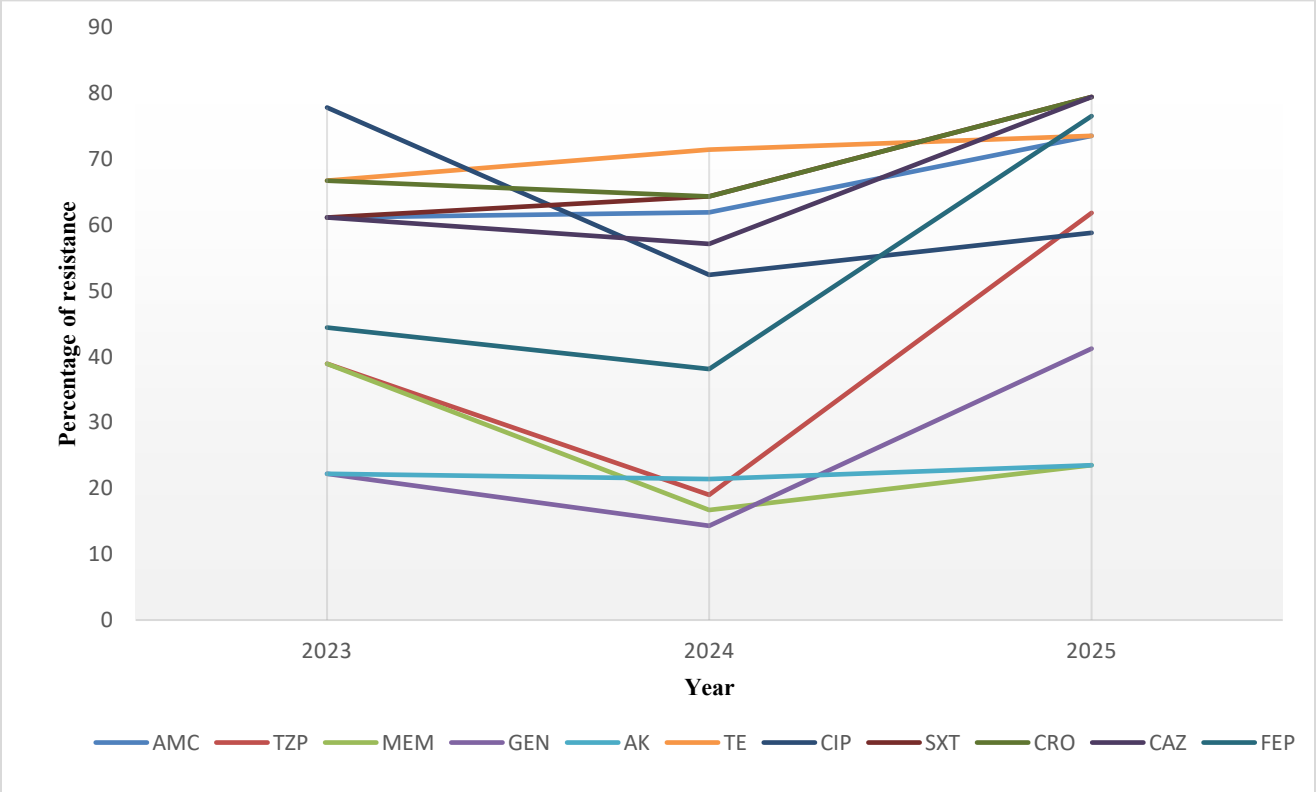


Figure 5: Antibiotic resistance trend of *K. pneumoniae* (2023-2025)

Figure 6 below depicts resistance trends of *Acinetobacter* species over the three years. Similar resistance trend was observed for all piperacilin-tazobactam, meropenem, and and gentamicin. The trend shows that 33.3%, 9.1% and 30% of *Acinetobacter* isolates were resistant to the above antibiotics in 2023, 2024 and 2025, respectively. Resistance to amikacin sharply dropped from 33.3% in 2023 to 0.0% in 2024 but increased to 10% in 2025. Most importantly, *Acinetobacter* isolates showed increased resistance to trimethoprim-sulfamethoxazole from 33.3% in 2023, 54.5% to 80.0% in 2025. About 50.0% of the isolates were resistant to third and fourth generation cephalosporin representatives in 2023 but showed fluctuating resistance patterns in 2024 and 2025.

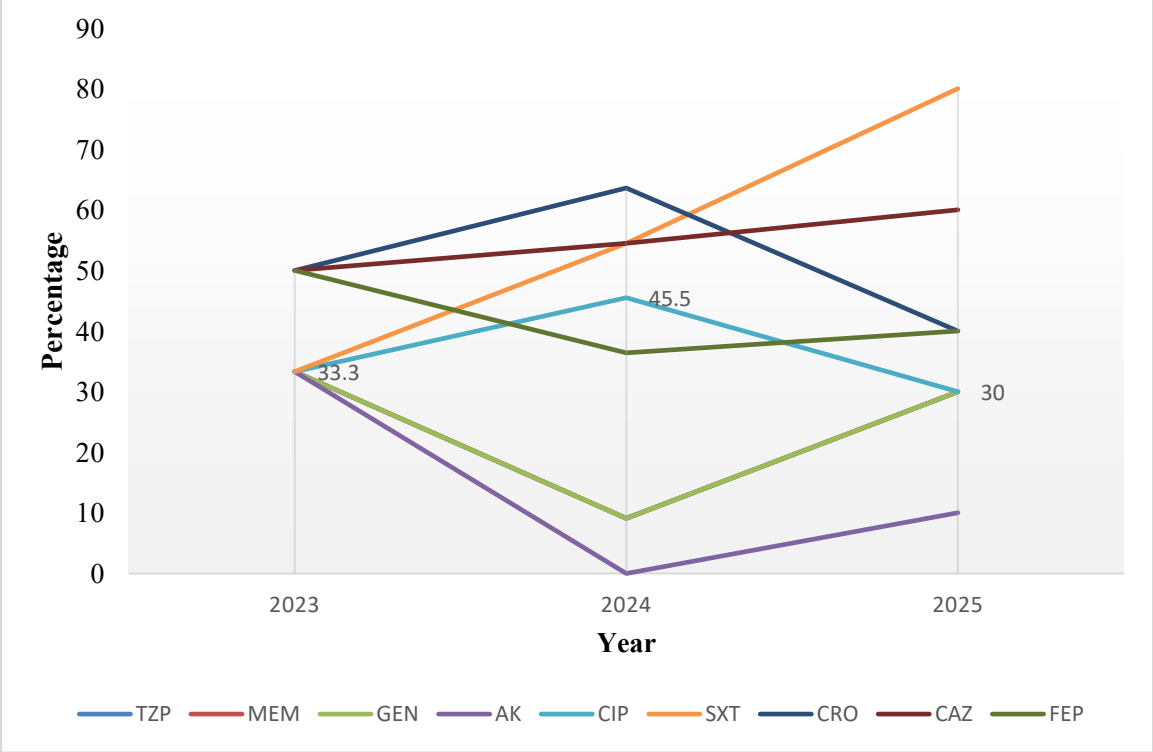


Figure 6: Antibiotic resistance trends of *Acinetobacter* species (2023-2025)

Figure 7 shows temporal antimicrobial resistance pattern of *Pseudomonas* species. The trend analysis shows that resistance to piperacillin-tazobactam was 37.5% in 2023, decreased markedly to 17.9% in 2024 but raised to 47.4% in 2025. In addition, resistance to meropenem was 50.0% in 2023 and raised to 57.1% and 71.1% in 2024 and 2025, respectively indicating a marked increase in antimicrobial resistance. Moreover, tobramycin resistance showed a marked and fluctuating trend, declining from 31.3% in 2023 to 10.7% in 2024, before sharply increasing to 52.6% in 2025. This substantial rebound in resistance may indicate changing antimicrobial selection pressure or the emergence and dissemination of resistant strains. Resistance to ciprofloxacin decreased from 50.0% in 2023 to 42.9% in 2024, but subsequently increased to 60.5% in 2025 indicating a concerning resistance trend. Resistance to third- and fourth-generation cephalosporins showed concerning and variable patterns over the study period.

Resistance to ceftazidime (third-generation cephalosporin) decreased markedly from 75.0% in 2023 to 42.9% in 2024, but increased again to 55.3% in 2025. In contrast, cefepime (fourth-generation cephalosporin) resistance increased progressively from 50.0% in 2023 to 53.8% in 2024 and 55.3% in 2025.

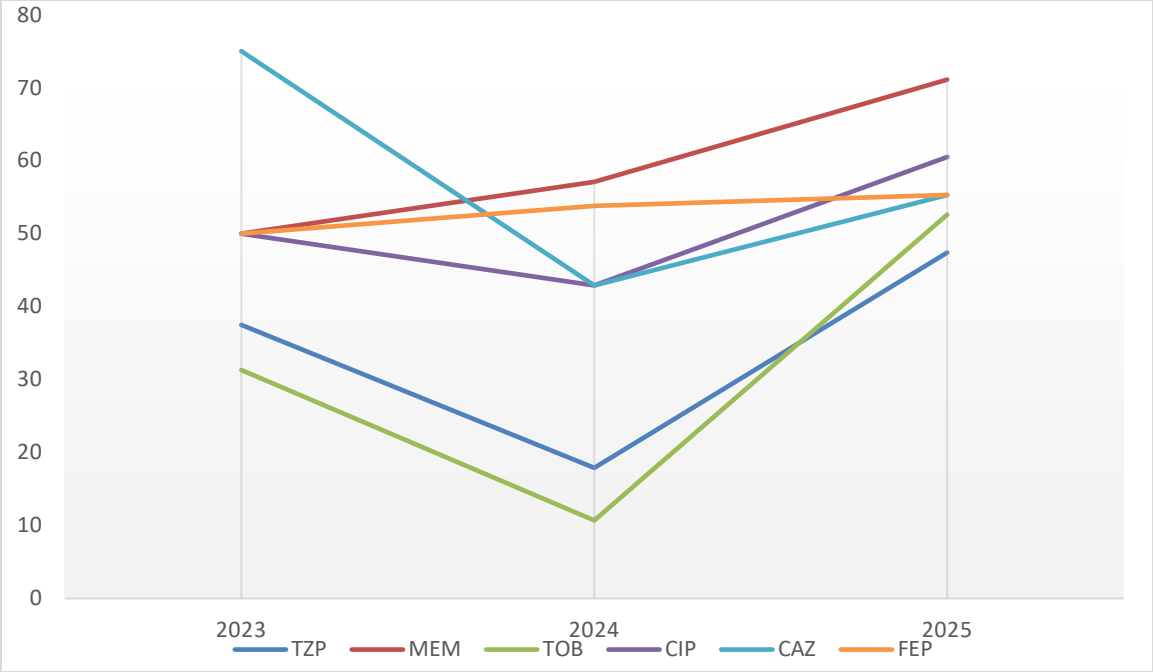


Figure 7: Antibiotic resistance trends of *Pseudomonas* species (2020-2025)

Enterobacter species is one of rarely isolated ESKAPE pathogens followed by *Enterococcus* species. Figure 7 depicts that resistance to piperacillin-tazobactam showed substantial year-to-year variation, increasing from 0% in 2023 to 66.7% in 2024, before declining to 25.0% in 2025. Despite the subsequent reduction, the marked increase observed in 2024 indicates considerable variability in piperacillin-tazobactam susceptibility and warrants continued surveillance. Moreover, meropenem, a carbapenem, showed a fluctuating resistance pattern during the study period. Resistance increased from 0.0% in 2023 to 33.3% in 2024, but declined to 0.0% in 2025. The absence of resistance in 2023 and 2025 is encouraging; however, the transient increase observed in 2024 warrants continued surveillance, particularly because carbapenems are important reserve agents for treating infections caused by multidrug-resistant Gram-negative pathogens. Resistance to aminoglycosides (gentamicin and amikacin) showed a fluctuating pattern over the study period. Resistance to both agents was 0% in 2023, increased to 33.3% in 2024, and subsequently declined in 2025, with gentamicin resistance remaining at 25% while amikacin resistance returned to 0%. In 2023 and 2024, almost all the *Enterobacter* isolates were resistant to trimethoprim-sulfamethoxazole, showing marked decrease to 50% in 2025. Resistance to cephalosporins was consistently high during the study period. Resistance to ceftriaxone, a third-

generation cephalosporin, remained at 100% in both 2023 and 2024, decreasing to 75.0% in 2025. Similarly, ceftazidime, another third-generation cephalosporin, showed 100% resistance in 2023 and 2024, followed by a decrease to 75.0% in 2025. In contrast, cefepime, a fourth-generation cephalosporin, showed a marked fluctuation, increasing from 0% resistance in 2023 to 100% in 2024 before declining to 25.0% in 2025 (Figure 8).

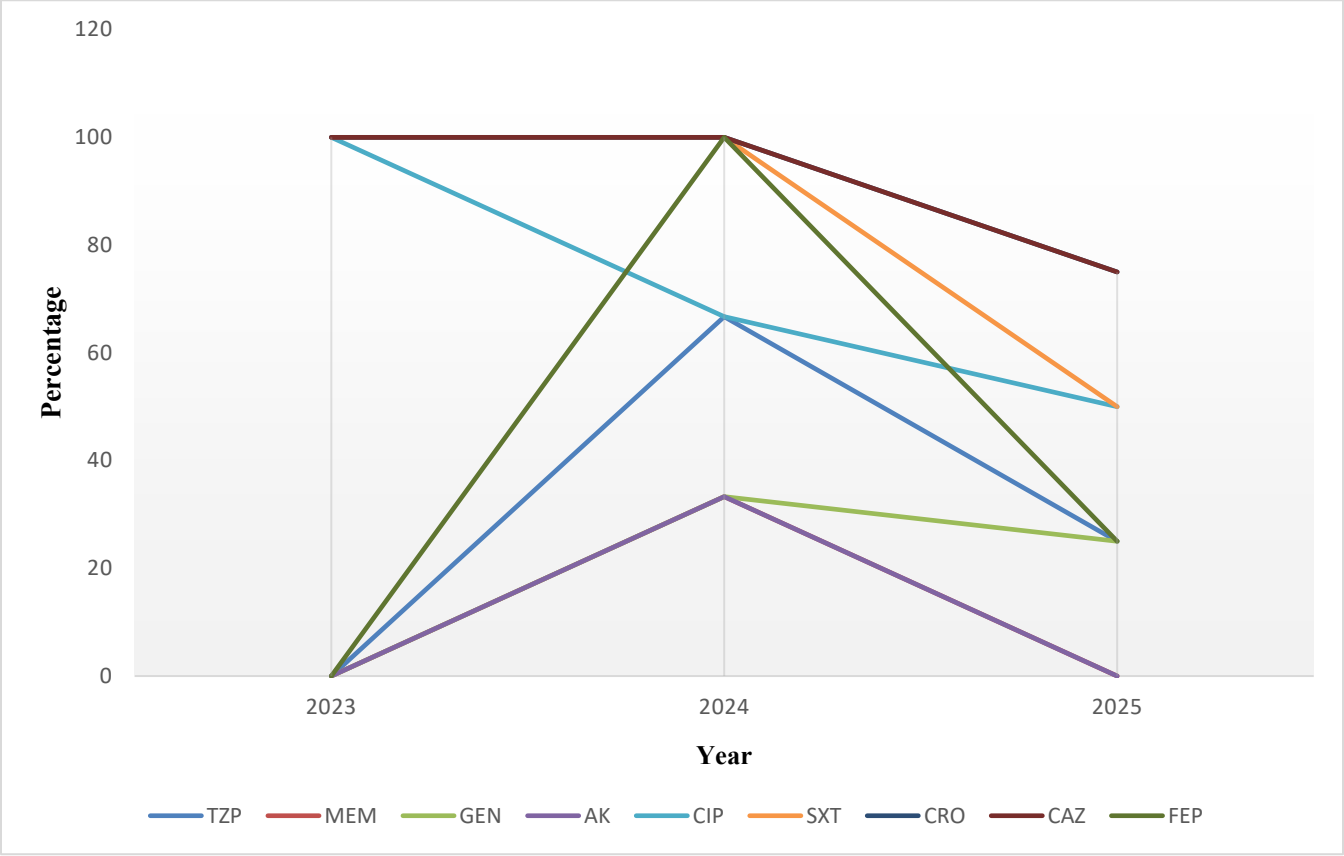


Figure 8: Antibiotic resistance trends of *Enterobacter* species (2023-2025)

Multiple Antibiotic Resistance Index (MARI) among ESKAPE Pathogens

According to this study, 464 (81.7%) of the ESKAPE pathogens exhibited MARI of greater than 0.2 and above. Three hundred eight (87.0%) of *S. aureus* isolates demonstrated an index of at least 0.2. Furthermore, 71 (25.5%) and 63 (76.8%) of *K. pneumoniae* and *Pseudomonas* species were found to have MAR index of 0.2 and above (Table 6).

Table 6: Multiple antibiotic resistance index of ESKAPE pathogens isolated from clinical specimens at Nekemte Public Health Research and Referral Laboratory Center, Nekemte, Ethiopia, 2023-2025

ESKAPE Pathogens	Number of isolates	MARI < 0.20, N (%)	MARI ≥ 0.20, N (%)
S. aureus	354	46 (13.0)	308 (87.0)
K. pneumoniae	94	23 (24.5)	71 (75.5)
Pseudomonas species	82	19 (23.2)	63 (76.8)
Acinetobacter species	27	13 (48.1)	14 (51.9)
Enterobacter species	9	1 (11.1)	8 (88.9)
Enterococcus species	2	2 (100)	0 (0.0)
Total	**568**	**104 (18.3)**	**464 (81.7)**

Distribution of MARI among Patient Characteristics

Of the total ESKAPE pathogens isolated from under five children, 121 (85.2%) had MARI ≥0.20. It was also found that about 122 (80.8%) of the pathogens that were recovered from patients aged ≥35 years had MARI ≥0.20. However, the index does not show significant difference across age groups (χ^2=2.340, p=0.674). This study revealed that the index showed significant differences across health facilities (χ^2 = 7.094, p=0.029), patient location (χ^2 = 3.965, p=0.046) and clinical specimens (χ^2 = 16.617, p=0.002) (Table 7).

Table 7: Distribution of MARI of ESKAPE pathogens among patient characteristics at Nekemte Public Health Research and Referral Laboratory Center, Nekemte, Ethiopia, 2023-2025

Variables	MARI ≥ 0.20	MARI < 0.20	Total	χ^2	*p*-value
Age (year)					
<5	121 (85.2)	21 (14.8)	142 (25.0)	2.340	0.674
5-14	66 (79.5)	17 (20.5)	83 (14.6)		
15-24	68 (78.2)	19 (21.8)	87 (15.3)		
25-34	87 (82.9)	18 (17.1)	105 (18.5)		
≥35	122 (80.8)	29 (19.2)	151 (26.6)		
Sex					
Male	230 (80.7)	55 (19.3)	285 (50.2)	0.374	0.541
Female	234 (82.7)	49 (17.3)	283 (49.8)		
Health facility type					
Hospital	413 (82.1)	90 (17.9)	503 (88.6)	7.094	**0.029***
Health center	7 (53.8)	6 (46.2)	13 (2.3)		
Clinic	44 (84.6)	8 (15.4)	52 (9.2)		
Patient location					
OPD	361 (80.0)	90 (20.0)	451 (79.4)	3.965	**0.046***
IPD	103 (88.0)	14 (12.0)	117 (20.6)		
Clinical specimens					
Middle ear discharge	284 (81.4)	65 (18.6)	349 (61.4)	16.617	**0.002***
Blood	63 (92.6)	5 (7.4)	68 (12.0)		
Urine	41 (80.4)	10 (19.6)	51 (9.0)		
Pus	62 (82.7)	13 (17.3)	75 (13.2)		
Others*	14 (56.0)	11 (44.0)	25 (4.4)		

***Significant at *p* <0.05** **OPD – Outpatient department IPD – Inpatient department**

Prevalence of Multidrug Resistance in ESKAPE Pathogens

Among the total ESKAPE pathogens isolated, 351 (61.8%) were multidrug resistant (MDR). The predominant multidrug resistant ESKAPE pathogen was found to be *S. aureus* (243, 69.2%) followed by *K. pneumoniae* (71, 20.2%) (Figure 9).

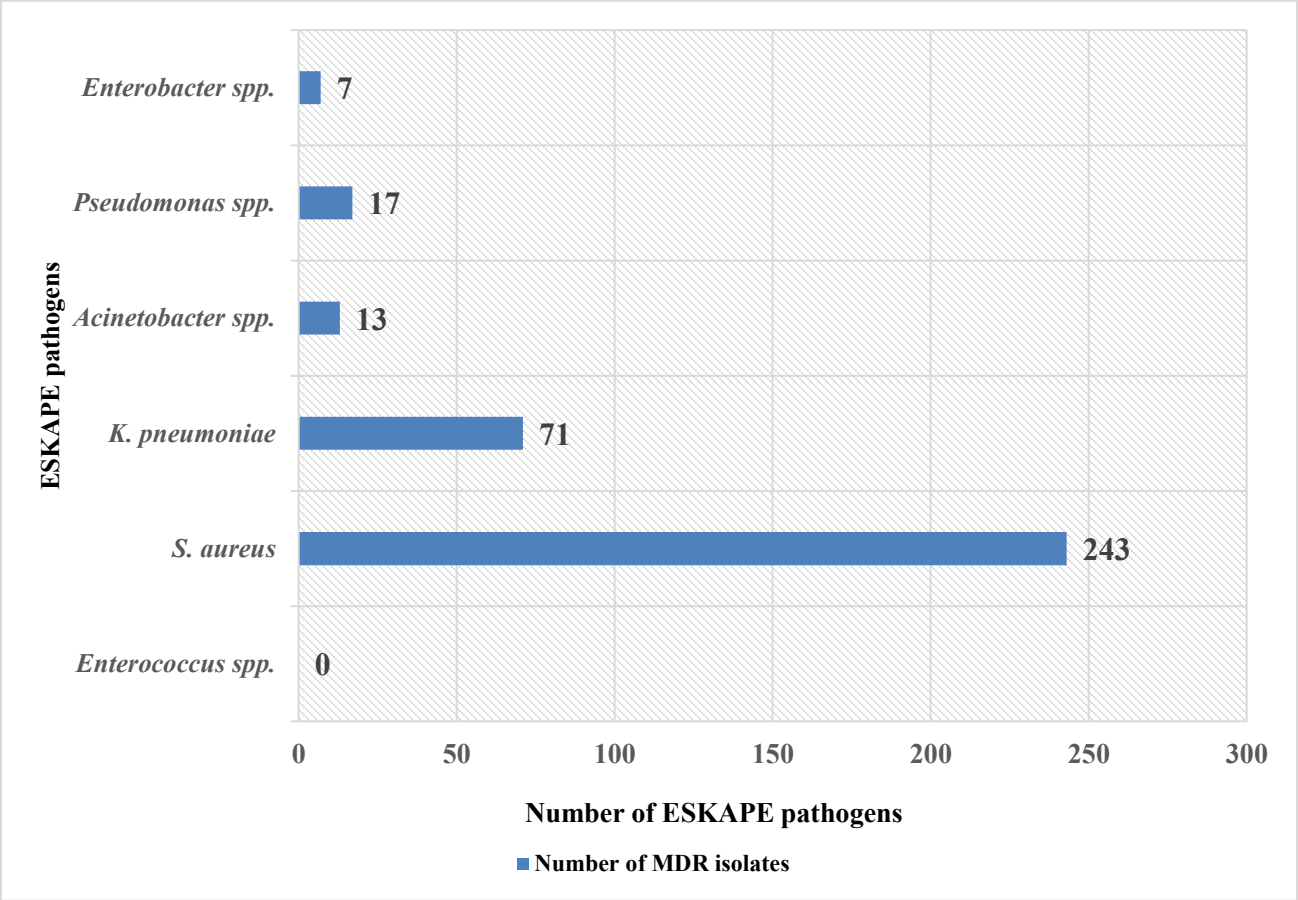


Figure 9: Prevalence of MDR ESKAPE pathogens from recovered from clinical specimens at Nekemte Public Health Research and Referral Laboratory Center, Nekemte, Ethiopia, 2023-2025

Trend analysis of MDR ESKAPE pathogens revealed the proportion of MDR isolates was 66.4% (97/146) in 2023, 58.4% (115/197) in 2024, and 61.8% (139/225) in 2025. Although the proportion of MDR isolates fluctuated over the three-year period, there was no statistically significant difference in MDR prevalence among years (χ^2 = 2.309, P = 0.315). Similarly, the Cochran–Armitage test for trend showed no significant linear trend in MDR prevalence across the study years (χ^2 = 0.547, P = 0.460) (Figure 10).

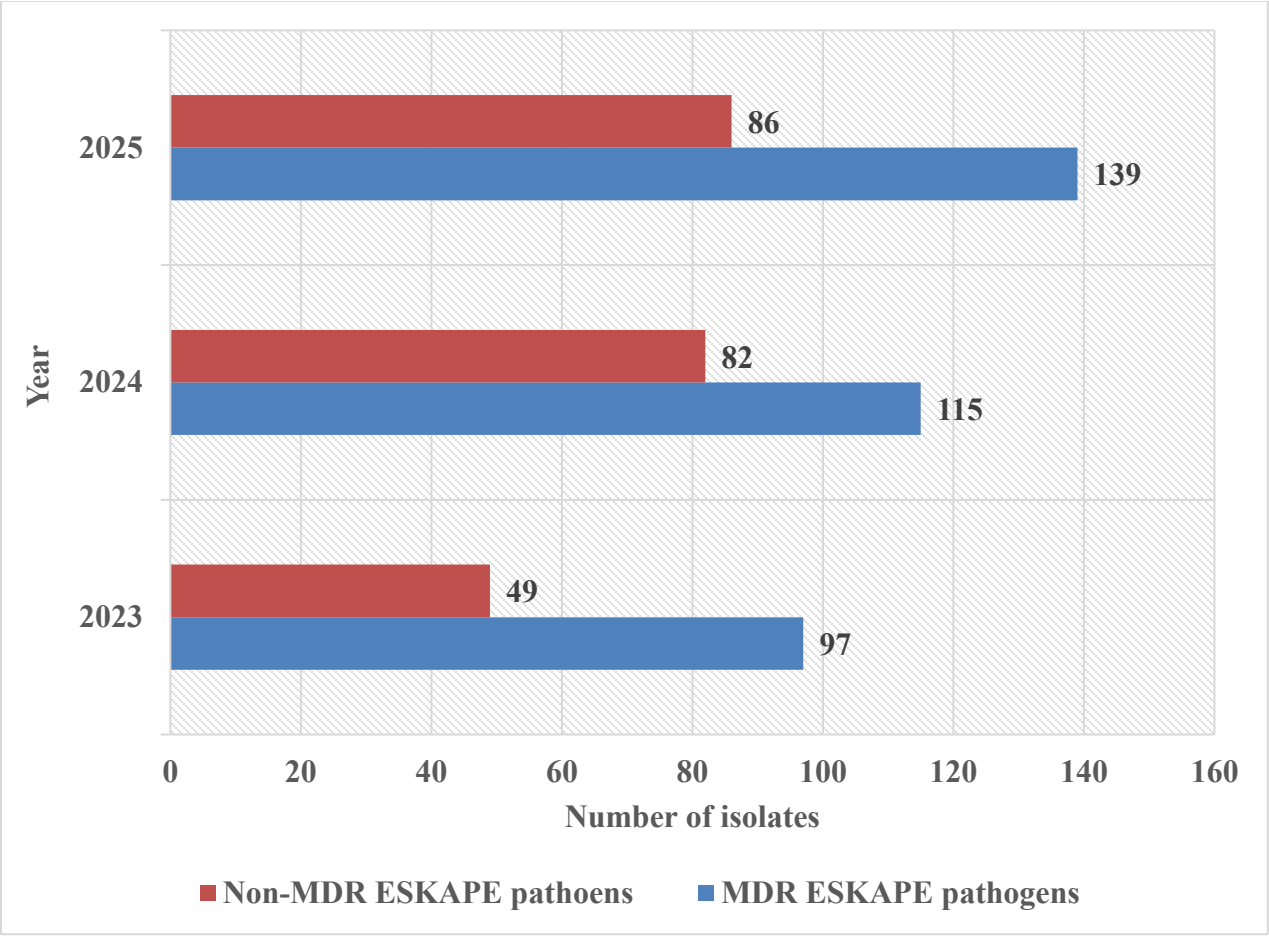


Figure 10: Temporal trend in the prevalence of multidrug-resistant (MDR) ESKAPE pathogens recovered from clinical specimens at Nekemte Public Health Research and Referral Laboratory Center, Nekemte, Ethiopia, 2023–2025

Distribution of MDR ESKAPE Pathogens among Patient Characteristics

According to this study, of the total ESKAPE pathogens recovered from under five, MDR accounted for 94 (66.2%). Moreover, 93 (61.1%) of the isolates from patients aged ≥35 years were MDR. AMONG 285 ESKAPE pathogens isolated from male patients, 171 (60.0%) were found to be MDR. As revealed by this study, the majority of isolates were recovered from hospitals (489/568, 86.0%), followed by clinics (53/568, 9.3%) and health centers (26/568, 4.6%). The proportion of MDR ESKAPE pathogens was 62.4% (305/489) in hospitals, 61.5% (16/26) in health centers, and 56.6% (30/53) in clinics. Although hospitals contributed the largest number of MDR isolates, the proportion of MDR isolates was comparable across the three facility types. Pearson's chi-square test showed no statistically significant association between health facility type and MDR status ($\chi^2 = 0.675$, $p = 0.714$), indicating that the distribution of MDR and non-MDR ESKAPE pathogens did not differ significantly by facility type.

The proportion of MDR ESKAPE pathogens was 59.4% (268/451) among OPD isolates and 70.9% (83/117) among IPD isolates. Although the majority of MDR isolates were obtained from the OPD because of the larger number of specimens, the proportion of MDR isolates was significantly higher among inpatients than outpatients ($\chi^2 = 5.219$, $p = 0.022$) (Table 8).

Table 8: Distribution of multidrug-resistant (MDR) and non-MDR ESKAPE pathogens by patient characteristics recovered from clinical specimens at Nekemte Public Health Research and Referral Laboratory Center, Nekemte, Ethiopia, 2023-2025

Variable	MDR status			χ^2	*p*-value
	MDR, N (%)	Not MDR, N (%)	Total, N (%)		
Age (year)					
<5	94 (66.2)	48 (33.8)	142 (25.0)	2.005	0.735
5-14	51 (61.4)	32 (38.6)	83 (14.6)		
15-24	50 (57.5)	37 (42.5)	87 (15.3)		
25-34	63 (60.0)	42 (40.0)	105 (18.5)		
≥35	93 (61.6)	58 (38.4)	151 (26.6)		
Sex					
Male	171 (60.0)	114 (40.0)	285 (50.2)	0.781	0.377
Female	180 (63.6)	103 (36.4)	283 (49.8)		
Health facility type					
Hospital	305 (62.4)	184 (37.6)	489 (86.1)	0.675	0.714
Health center	16 (61.5)	10 (38.5)	26 (4.6)		
Clinic	30 (56.6)	23 (43.4)	53 (9.3)		
Location					
OPD	268 (59.4)	183 (40.6)	451	5.219	**0.022***
IPD	83 (70.9)	34 (29.1)	117		
Type of clinical specimen					
Ear discharge	207 (59.3)	142 (40.8)	349	9.337	0.053
Blood	52 (76.5)	16 (23.5)	68		
Urine	33 (64.7)	18 (35.3)	51		
Pus	47 (62.7)	28 (37.3)	75		
Others**	12 (48.0)	13 (52.0)	25		

***Significant at $p<0.05$** **Body fluids, sputum, CSF, nasal swab

Discussion

ESKAPE pathogens are major causes of healthcare-associated infections and are hard to treat because they can become resistant to antibiotics. In this study ESKAPE pathogens made up a part of the bacteria found in patient samples in Western Oromia, Ethiopia. The bacteria showed a lot of differences in how they responded to different medicines over time. This means that there are a lot of infections that are hard to treat with medicine in this area. We need to keep watching for bacteria that're resistant to medicine and make sure we use antibiotics wisely and take steps to prevent infections, in this region. ESKAPE pathogens are a concern and we need to take action to stop them from spreading.

In this study, the overall prevalence of ESKAPE pathogens was found to be 59.5% (95% C: 56.4-62.6) indicating that nearly six out of every ten bacterial isolates recovered from clinical specimens belonged to the ESKAPE group. The prevalence was statistically significant ($p< 0.001$), highlighting the substantial burden of clinically important ESKAPE pathogens in the study setting and underscoring the need for strengthened antimicrobial stewardship, infection prevention and control, and routine AMR surveillance. The current prevalence of ESKAPE pathogen is higher than that reported from multicenter study conducted in Addis Ababa 28% (24), Tanzania 43.3% (25), Rwanda 30% (26) and Turkey 24.9% (27) but lower than the study findings from ALERT hospital 70.4% (28), Felege Hiwot Comprehensive Specialized Hospital 67% (29), Uganda 84.1% (30), Brazil 68.7% (31), and Romania 75.6% (32). Variations in study population, clinical specimens, healthcare settings, geographical and temporal contexts, and patterns of antimicrobial exposure may explain the variation in ESKAPE pathogen prevalence observed across studies. Differences in sampling strategies, sample size, laboratory identification methods and AST standards may also contribute to the observed variability.

The prevalence of ESKAPE pathogens displayed a varying trend throughout the study duration, rising from 56.5% in 2023 to 65.0% in 2024, then decreasing to 57.1% in 2025. While the prevalence reached its peak in 2024, the overall variation over the three years was not statistically significant ($\chi^2 = 5.750$, $p = 0.056$). This indicates that, although there seems to be a rise in ESKAPE pathogen prevalence in 2024, the year-to-year changes observed may be due to random variation instead of a steady temporal trend. Nonetheless, the consistently elevated prevalence observed during the study emphasizes the significant role of ESKAPE pathogens in the bacterial burden and reinforces the necessity for ongoing antimicrobial resistance monitoring, enhanced infection prevention and control strategies, and effective antimicrobial stewardship efforts. Other study findings revealed varied trends of ESKAPE pathogens (33,34).

This study revealed that the prevalence of ESKAPE pathogens indicated significant differences across health facilities (p = 0.030) with hospitals being the main source. The differences in the distribution of ESKAPE pathogens among health facility types may be explained the differences in patient complexity, referral patterns, antimicrobial use, diagnostic capacity, infection prevention and control practices and healthcare environment exposure. Evidences indicated that substantial variation in the prevalence and composition of ESKAPE pathogens across healthcare settings supports the importance of local surveillance rather than assuming uniform distribution (35). Moreover, it was also found that the distribution of ESKAPE pathogens showed significant differences across patient locations (p = 0.003). This may reflect differences in the healthcare exposure, disease severity, exposure to antibiotics, length of hospitalization.

in the present study may reflect differences in healthcare exposure, disease severity, antimicrobial exposure, and duration of hospitalization. ESKAPE organisms are strongly associated with healthcare-associated infections and are particularly important among hospitalized and critically ill patients, where prolonged hospitalization, invasive procedures, previous antibiotic exposure, and intensive-care admission provide opportunities for acquisition and transmission. ESKAPE pathogens are particularly important among hospitalized patients where prolonged hospitalization, invasive procedures, previous antibiotic exposure, and intensive-care admission provide opportunities for acquisition and transmission (36–38). In contrast, their occurrence among outpatient departments shows that they are not restricted to hospital-acquired infections and may also circulate in the community or be introduced through previous healthcare exposure (39).

The distribution of ESKAPE pathogens by clinical specimens has also an epidemiological importance because they have different clinical niches and are associated with particular infection syndromes. In the present study, the most common source of clinical specimen for the pathogens was middle ear discharge. It was found that significant difference was observed in the distribution of ESKAPE pathogens among clinical specimens ($p < 0.001$). This findings are also consistent with other similar studies elsewhere in the world (28,29). In the present study, middle ear discharge was the predominant source of ESKAPE isolates. The predominance of ESKAPE pathogens in middle ear discharge may reflect the substantial contribution of chronic or recurrent otitis media, repeated antimicrobial exposure, and prolonged or inappropriate antibiotic use, which can select for resistant organisms.

In this study, the predominant ESKAPE pathogen was *S. aureus* 62.3% followed by *K. pneumoniae* 16.5% and *Pseudomonas* species 14.4%. Similarly, Ethiopian study at ALERT hospital identified S. aureus and K. pneumoniae as predominant isolates with frequencies of 53.4% and 22.4%, respectively (28), but study from Felege Hiwot Specialized Hospital reported K. pneumoniae as

predominant isolated (29). Moreover, systematic and meta-analysis shows predominance of *S. aureus* accounting for 79.5% followed by *A. baumannii* 27.6% (35). Another review revealed that and *S. aureus* 23.1% and *K. pneumoniae* 20.9% were predominant isolates (40). The variation in the prevalence of predominant ESKAPE pathogens across studies may be attributed to differences in patient populations, clinical conditions, specimen types, healthcare settings, geographic locations, antimicrobial-use patterns, infection-prevention practices, and study periods. In addition, differences in sampling strategies, sample size, bacterial identification methods, and local circulating strains may contribute to the variability observed across studies.

Antimicrobial susceptibility testing revealed that 83.1% and 61.9% of *S. aureus* isolates were found to be sensitive to gentamicin and clindamycin, respectively while important resistance was observed with penicillin 96.0% and cefoxitin 65.0%. On the other hand, the predominant Gram-negative ESKAPE bacteria, *K. pneumoniae* showed highest susceptibility to amikacin 74.5%, gentamicin 72.3% and highest resistance to ceftriaxone 79.2%, tetracycline 71.3% and trimethoprim-sulfamethoxazole 69.1%. Moreover, piperacillin-tazobactam was found to be an antibiotic to which 64.5% of *Pseudomonas* species were sensitive while 62.2% of them were resistant to meropenem. Majority of *Acinetobacter* species 81.5% where sensitive to amikacin and 77.8% of them were sensitive to both piperacillin-tazobactam and gentamicin. Different studies from different settings reported varying susceptibility patterns with varying rates (24,29,30,41,42). The observed variability in AST profiles highlights the heterogeneous nature of antimicrobial resistance among ESKAPE pathogens. Rather than indicating inconsistency, these differences may reflect genuine local variation in resistance epidemiology combined with differences in study populations and laboratory methodology. The findings therefore support the importance of continuous, facility- and region-specific AMR surveillance, routine AST, and antimicrobial stewardship to ensure that empirical treatment recommendations remain consistent with the prevailing local resistance patterns.

The analysis of MAR index revealed that 81.7% of the pathogens demonstrated MAR index ≥0.20 with *S. aureus*, *Pseudomonas* species, *K. pneumoniae*, and *Acinetobacter* species being the predominant isolates with the highest indices accounting for 87.0%, 76.8%, 51.9%, respectively. The high MARI observed in the present study is particularly concerning because ESKAPE pathogens are important causes of healthcare-associated and difficult-to-treat infections. A high MARI >0.20, indicates a substantial multidrug-resistance burden and possible antimicrobial selection pressure. This may limit empirical treatment options and increase treatment failure and healthcare costs. The finding highlights the need for culture- and AST-guided therapy, strengthened antimicrobial stewardship, IPC, and continuous local AMR surveillance (43). Furthermore, it was also found that the distribution of ESKAPE pathogens indicated significant differences across health facility types

(p=0.029), patient location (p=0.046) and clinical specimens (p=0.002). Study from Zimbabwe showed similar findings (44).

The overall MDR among ESKAPE pathogens in the present study was 61.8% (57.7-65.7) with *S. aureus* 69.2% and *K. pneumoniae* 20.2% being predominant isolates. This finding underscores high AMR burden, reflecting the ability of ESKAPE organisms to acquire mechanisms that bypass standard clinical therapeutics. A study from southern Ethiopia reported 84.3%, which was higher than the current study finding (45). Another study revealed that 95% of *S. aureus* and *K. pneumoniae* were MDR (28). In another study, the MDR rate was reported to be 90.5%, with *A. baumannii* accounting for 95.6% (41). MDR rates may vary considerably across studies because of differences in study population, healthcare setting, specimen type, geographic location, study period, bacterial species distribution, prior antibiotic exposure, and local antimicrobial-prescribing and infection-control practices. In this study, ESKAPE pathogen distribution differed significantly between OPD and IPD patients (p=0.022) likely due to greater healthcare exposure among inpatients. Prolonged hospitalization, prior antibiotic use, invasive procedures, and healthcare-associated transmission may increase ESKAPE acquisition among inpatients. In contrast, OPD patients have greater community exposure and less healthcare-associated risk.

Strength and limitation

The strength of this study is its inclusion of clinical isolates and several ESKAPE pathogens. This study allows an assessment of prevalence, antimicrobial susceptibility, MDR patterns and MARI across different patient characteristics. This study also has some limitations. Because this study is retrospective it relied on laboratory records so it could not evaluate risk factors such as prior antibiotic use, hospitalization history or underlying conditions. Additionally, differences in specimen types, antimicrobial panels and AST practices may affect comparisons with studies. The findings of this study may not be fully generalizable to healthcare facilities, outside the study area.

Conclusion

This study indicated a considerable prevalence of ESKAPE pathogens, with significant variation in their distribution by health facility type, patient location and clinical specimens.

Moreover, a high proportion of ESKAPE isolates were found to be MDR, with MDR distribution varying significantly across patient location. In addition, the high proportion of isolates with MARI >0.20 further indicated a substantial MDR burden and possible antimicrobial selection pressure. These findings underscore the need to strengthen routine culture and AST services, promote diagnostic-guided antibiotic therapy and antimicrobial stewardship, strengthen infection prevention and control, and maintain continuous facility-specific AMR surveillance to guide empirical

treatment and limit the spread of resistant ESKAPE pathogens.

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Author contribution

ETG: Conceptualization, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. AD: Methodology, Writing – review & editing. KT: Data curation, Writing – review & editing. KB: Writing – review & editing. DJ: Project administration, Resources.

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Data availability statement

The original contributions presented in the study are included in the article. Additional documents are available from the corresponding author on reasonable request.

Declaration

Ethics statement

Permission was obtained from Nekemte Public Health Research and Referral Laboratory Center (Ref. Number: NPHRRL-2543/4/18). The requirement for obtaining informed consent was waived owing to the retrospective nature of the study. All authors have provided consent for the publication of the manuscript.

Competing interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Low JA, Pang JS, Lim TCM, Parimannan S, Rajandas H, Chuah C. ESKAPE pathogens and antimicrobial resistance: emerging trends and therapeutic challenges. Future Microbiol [Internet]. 2026 Apr 13;21(6):585–99. Available from: https://doi.org/10.1080/17460913.2026.2677420

2. Baekkeskov E, Pierre J. More than medicine: antimicrobial resistance (AMR) as a social and political challenge that can be overcome. J Eur Public Policy. 2024;31(12):3941–56.

3. Miller WR, Arias CA. ESKAPE pathogens: antimicrobial resistance, epidemiology, clinical impact and therapeutics. Nat Rev Microbiol [Internet]. 2024;22(10):598–616.

4. Krishnaprasad VH, Kumar S. Antimicrobial Resistance: An Ultimate Challenge for 21st Century Scientists, Healthcare Professionals, and Policymakers to Save Future Generations. J Med Chem. 2024;67(18):15927–30.

5. Dadgostar P. Antimicrobial resistance: implications and costs. Infect Drug Resist. 2019;12:3903–10.

6. Sajerli B, Sarkadi-Nagy Á, Burián K, Orosz L. Two decades of ESKAPE pathogens: Longitudinal analysis of antibiotic resistance trends between 2002 and 2024 in a Hungarian clinical center. Epidemiol Infect. 2026;154(347):1–13.

7. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, et al. Bad bugs, no drugs: No ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis. 2009;48(1):1–12.

8. Rice LB. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: No ESKAPE. J Infect Dis. 2008;197(8):1079–81.

9. Organiation WH. WHO Bacterial Priority Pathogens List , 2024. 2024. 1–72 p.

10. De Oliveira DMP, Forde BM, Kidd TJ, Harris PNA, Schembri MA, Beatson SA, et al. Antimicrobial resistance in ESKAPE pathogens. Clin Microbiol Rev. 2020;33(3):1–49.

11. Loomba P, Taneja J, Mishra B. Methicillin and vancomycin resistant S. aureus in hospitalized patients. J Glob Infect Dis. 2010;2(3):275.

12. Mansournia, M. A., Higgins, J. P. T., Sterne, J. A. C., & Hernan MA. Resistance in Vancomycin-Resistant Enterococci. Physiol Behav. 2017;176(10):139–48.

13. Takkonelli Evelina, Carrara Elena, Savoldi Alessia, Harbarth Stephan, Mendelson Marc, Monnet L Dominique, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis. 2018;18(3):318–27.

14. Karakonstantis S, Kritsotakis EI, Gikas A. Pandrug-resistant gram-negative bacteria: A systematic review of current epidemiology, prognosis and treatment options. J Antimicrob Chemother.

2020;75(2):271–82.

15. Sakalauskienė GV, Malcienė L, Stankevičius E, Radzevičienė A. Unseen Enemy: Mechanisms of Multidrug Antimicrobial Resistance in Gram-Negative ESKAPE Pathogens. Antibiotics. 2025;14(1):1–43.
16. Mulani MS, Kamble EE, Kumkar SN, Tawre MS, Pardesi KR. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: A review. Front Microbiol. 2019;10:1–24.
17. Daruka L, Czikkely MS, Szili P, Farkas Z, Balogh D, Grézal G, et al. ESKAPE pathogens rapidly develop resistance against antibiotics in development in vitro. Vol. 10, Nature Microbiology. 2025. 313–331 p.
18. Holmes AH, Moore LSP, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, et al. Understanding the mechanisms and drivers of antimicrobial resistance. Lancet. 2016;387(10014):176–87.
19. WHO Regional Office for Europe. WHO Regional Office for Europe and European Centre for Disease Prevention and Control. Surveillance of antimicrobial resistance in Europe, 2022 data: executive summary. Copenhagen: Copenhagen WHO Reg Off Eur 2023 [Internet]. 2023;1–8. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/Nov2023-ECDC-WHO-Executive-Summary.pdf
20. Cassini A, Högberg LD, Plachouras D, Quattrocchi A, Hoxha A, Simonsen GS, et al. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. Lancet Infect Dis. 2019;19(1):56–66.
21. Wyres KL, Lam MMC, Holt KE. Population genomics of Klebsiella pneumoniae. Nat Rev Microbiol [Internet]. 2020;18(6):344–59.
22. Camacho-Ortiz A, Flores-Treviño S, Bocanegra-Ibarias P. Prevalence of difficult-to-treat resistance in ESKAPE pathogens in a third level hospital in Mexico. Infect Prev Pract. 2025;7(1):1.
23. Teshome A, Alemayehu T, Deriba W, Ayele Y. Antibiotic Resistance Profile of Bacteria Isolated from Wastewater Systems in Eastern Ethiopia. J Environ Public Health. 2020;1–10.
24. Hailemichael EB, Desta AF, Taye G, Gari SR, Adamu E, Ayenew Z, et al. Prevalence, microbiological profiles, and determinants of hospital-acquired pneumonia in Addis Ababa: A focus on Pseudomonas aeruginosa and its antimicrobial resistance patterns in three hospitals. PLoS One [Internet]. 2026;21(1 January):1–15.
25. Augustine FF, Mgaya XM, Yahya SA, Niccodem EM, Matee MI. An alarming prevalence of

multidrug-resistant (MDR) ESKAPE pathogens and other drug-resistant bacteria isolated from patients with bloodstream infections hospitalized at Muhimbili National Hospital in Dar es Salaam, Tanzania. Ger J Microbiol. 2023;3(3):7–15.

26. Muhinda C, Murenzi G, Al-Hassan L, Seruyange E, Mutesa L, Gylfe Å. High Antimicrobial Resistance in ESKAPE Pathogens at a Rwandan Tertiary Hospital. Pathogens. 2025;14(12):1–11.
27. Orhan Z, Kirişci Ö, Doğaner A, Altun M, Küçük B, Aral M. Antibiotic Resistance Trends in ESKAPE Pathogens Isolated at a Health Practice and Research Hospital: A Five-Year Retrospective Study. J Infect Dev Ctries. 2024;18(12):1899–908.
28. Gebeyehu R, Beyene GT, Mekonnen M, Lema T, Getu Y, Worku J, et al. Prevalence and antimicrobial resistance of ESKAPEE pathogens at ALERT Hospital, Addis Ababa, Ethiopia: A one-year retrospective study. Microbe (Netherlands). 2026;10:100665.
29. Erkihun M, Assefa A, Legese B, Almaw A, Berhan A, Getie B, et al. Epidemiology and Antimicrobial Resistance Profiles of Bacterial Isolates from Clinical Specimens at Felege Hiwot Comprehensive Specialized Hospital in Ethiopia: Retrospective Study. Bacteria. 2024;3(4):405–21.
30. Bazira J, Nalumaga PP, Quraishi B, Walekhwa AW, Lawrence M, Iramiot JS. Trends of Antibiotic Resistance in ESKAPE Pathogens in Mbarara Regional Referral Hospital (2015–2022), South Western, Uganda. Can J Infect Dis Med Microbiol. 2025;2025(1):129.
31. de Paula L, Lopes GFM, Santos FR da S, Oliveira ALB, de Pilla Varotti F, da Cruz Nizer WS, et al. Prevalence of ESKAPEE pathogens as agents of healthcare-related infection in Brazil. Microbe (Netherlands). 2025;7:100326.
32. Bereanu AS, Bereanu R, Mohor C, Vintilă BI, Codru IR, Olteanu C, et al. Prevalence of Infections and Antimicrobial Resistance of ESKAPE Group Bacteria Isolated from Patients Admitted to the Intensive Care Unit of a County Emergency Hospital in Romania. Antibiotics. 2024;13(5).
33. Ibrahim AH, Adan HAA, Idiris MH, Asir SM, Mohamud SM, Nor LA, et al. Antimicrobial resistance trends of ESKAPEE pathogens isolated from intensive care unit patients at a tertiary care hospital in Mogadishu, Somalia: a retrospective study (2020–2024). Front Microbiol. 2026;17:1–13.
34. Sajerli B, Sarkadi-nagy Á, Burián K, Orosz L. Two decades of ESKAPE pathogens : Longitudinal analysis of antibiotic resistance trends between 2002 and 2024 in a Hungarian clinical centre. Epidemiol Infect. 2026;
35. Khasapane NG, Nkhebenyane SJ, Lekota K, Thekisoe O, Ramatla T. “One Health” Perspective on Prevalence of ESKAPE Pathogens in Africa: A Systematic Review and Meta-Analysis.

Pathogens. 2024;13(9).

36. Woh PY, Zhang X. The burden of ESKAPE pathogen-related hospital-acquired infections: clinical and financial perspective from a systematic review. J Hosp Infect [Internet]. 2025;162:377–85. Available from: https://www.sciencedirect.com/science/article/pii/S0195670125001793

37. Guzek A, Tomaszewski D, Rybicki Z, Piechota W, Mackiewicz K, Konior M, et al. ESKAPE Pathogens in Bloodstream Infections: Dynamics of Antimicrobial Resistance from 2018 to 2024—A Single-Center Observational Study in Poland. J Clin Med. 2025;14(19):1–21.

38. Gaber SN, Bassyouni RH, Wegdan AA, Abd-elrahman HS, Elkhateeb AF, Botros JM, et al. Prevalence, resistance profile of ESKAPEE pathogens, and molecular detection of PMQR-associated genes among Levofloxacin-resistant gram-negative ESKAPEE isolates from critically-ill patients. BMC Infect Dis. 2026;26(1).

39. Sah RK, Bhattarai A, Khadka P, Sharma S, Mishra SK, Rai JR, et al. Current Antibiotic Resistance Profile of ESKAPE Pathogens in a Nepalese Hospital: A Cross-Sectional Study. Can J Infect Dis Med Microbiol. 2025;2025(1).

40. Khasapane NG, Nkhebenyane SJ, Lekota KE, Thekisoe O, Ramatla T. An insight into the antibiotic resistance profiles of ESKAPE pathogens isolated from humans in Africa: a systematic review and meta-analysis. BMC Infect Dis. 2025;25(1).

41. Abukhalil AD, Barakat SA, Mansour A, Al-Shami N, Naseef H. ESKAPE Pathogens: Antimicrobial Resistance Patterns, Risk Factors, and Outcomes a Retrospective Cross-Sectional Study of Hospitalized Patients in Palestine. Infect Drug Resist. 2024;17:3813–23.

42. Pandey R, Mishra SK, Shrestha A. Characterisation of eskape pathogens with special reference to multidrug resistance and biofilm production in a nepalese hospital. Infect Drug Resist. 2021;14:2201–12.

43. Adijat JA, S OM, B OL, B OA, O PG. Multiple Antibiotic Resistance Index of Escherichia Coli Isolates in a Tertiary Hospital in South-West Nigeria. Med J Zambia. 2017;44(4):225–32.

44. Hetsa BA, Asante J, Amoako DG, Abia ALK, Mbanga J, Essack SY. Multidrug-Resistant ESKAPEEc Pathogens From Bloodstream Infections in South Africa: A Cross-Sectional Study Assessing Resistance to WHO AWaRe Antibiotics. Heal Sci Reports. 2025;8(6):1–13.

45. Seid M, Bayou B, Aklilu A, Tadesse D, Manilal A, Zakir A. Antimicrobial resistance patterns of WHO priority pathogens at general hospital in Southern Ethiopia during the COVID-19 pandemic , with particular reference to ESKAPE-group isolates of surgical site infections. BMC Microbiol. 2025;25(84):1–14.