Ethiopian Journal of Health and Biomedical Sciences
Online ISSN: 2790-3087

Original article
Open access

Prevalence and Antimicrobial Resistance Patterns of Gram-Negative Bacteria at Amhara Public Health Institute, Northwest Ethiopia: A Six-Year Retrospective Study

Alemayehu Abate[1]*, Mickel Geyie[2], Gizeaddis Belay[2], Desalew Salew[1]

[1]Health Research Development Directorate, Amhara Public Health Institute, Bahir Dar, Ethiopia
[2]Bacteriology Reference Laboratory, Amhara Public Health Institute, Bahir Dar, Ethiopia

***Correspondence:** *alexu2love@gmail.com*

ABSTRACT

Background: The global rise of multidrug-resistant Gram-negative bacteria presents a major public health challenge, particularly in low- and middle-income countries. Limited antimicrobial resistance surveillance data in Ethiopia hampers evidence-based treatment and policy decisions.

Objective: This study assessed the prevalence and antimicrobial resistance patterns of Gram-negative bacteria isolated over six years (2018–2024) at the Amhara Public Health Institute (APHI), Northwest Ethiopia.

Methods: An institution-based retrospective study was conducted using archived microbiology laboratory data from January 2018 to December 2024. All culture-confirmed Gram-negative bacterial isolates with complete antimicrobial susceptibility test results were included. Antimicrobial susceptibility test was performed using the Kirby–Bauer disk diffusion method according to the CLSI guidelines. Data were analyzed SPSS version 26 software. Chi-square tests were used to assess the associations between outcome and demographic variables. Statistical significance was declared at $p < 0.05$.

Results: A total of 350 Gram-negative isolates were analyzed. Escherichia coli (29.4%, 95% CI: 24.6–34.2) and Klebsiella spp. (27.7%, 95% CI: 23.0–32.4) were the predominant isolates. Overall, 53.2% of isolates were classified as MDR. Highest resistance rates were observed against chloramphenicol (97.3%), sulfonamides (80.3%), and ampicillin (82.4%). The isolates showed the lowest resistance against Carbapenems (24.9%). Age ($\chi^2 = 106.69$, $p < 0.001$) and sex ($\chi^2 = 12.86$, $p = 0.045$) were significantly associated with multidrug resistance bacterial infection.

Conclusion: More than half of Gram-negative isolates were multidrug resistant in the study area. Strengthening AMR surveillance, antimicrobial stewardship programs, and routine culture-based diagnosis is urgently required.

Keywords: Multidrug resistance; Gram-negative bacteria; Antimicrobial resistance; Ethiopia; Bahir Dar.

Article History: Received: July 18, 2025 Revised: October 14, 2025 Accepted: December 17, 2025

Citation: Alemayehu A. Mickel G. Gizeaddis B. Desalew S. Prevalence and Antimicrobial Resistance Patterns of Gram-Negative Bacteria at Amhara Public Health Institute, Northwest Ethiopia: A Six-Year Retrospective Study. Ethiop J Health Biomed Sci. 2025;15(2):45-53. https://doi.org/10.20372/ejhbs.1129

INTRODUCTION

Antimicrobial resistance (AMR) among Gram-negative bacteria has increased substantially over the past decade and is now recognized as a major global public health threat (1). These organisms have developed resistance to multiple commonly used antibiotics, resulting in the emergence of so-called "superbugs," which significantly complicate the treatment of bacterial infections (2). Multidrug resistance (MDR) is defined as resistance to at least one antimicrobial agent in three or more antibiotic classes, often mediated by the acquisition of multiple resistance genes and mechanisms (3).

Gram-negative bacteria, particularly members of the *Enterobacteriaceae* such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter* species, as well as non-fermenting organisms including *Pseudomonas aeruginosa* and *Acinetobacter* species, are among the leading causes of MDR infections worldwide (4). According to the first comprehensive global assessment, bacterial AMR was associated with approximately 4.95 million deaths in 2019, including 1.27 million deaths directly attributable to resistant infections. The burden of AMR is disproportionately higher in low- and middle-income countries due to limited diagnostic capacity, weak surveillance systems, and widespread inappropriate antibiotic use (5).**Top of Form**

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Evidence from developing countries indicates that Gram-negative bacteria exhibit high resistance rates to commonly prescribed antibiotics, thereby reducing treatment effectiveness and increasing morbidity, mortality, and healthcare costs (6). In Ethiopia, a systematic review and meta-analysis reported a pooled MDR prevalence of 70.5%, highlighting the magnitude of the problem and the urgent need for effective interventions (7). To address this global health threat, the World Health Organization (WHO) recommends strengthening antimicrobial stewardship programs, enhancing surveillance systems, and promoting rational antibiotic use (8).

Routine epidemiological surveillance of antimicrobial resistance is essential to monitor trends, guide empirical treatment, and inform infection prevention and control strategies. Establishing robust surveillance systems and generating reliable local data are critical components of national and global AMR control efforts (9). However, in many healthcare settings in Ethiopia, comprehensive data on the prevalence and antimicrobial susceptibility patterns of Gram-negative bacterial isolates remain limited.

The lack of local epidemiological evidence hinders evidence-based clinical decision-making, contributes to inappropriate empirical antibiotic use, and accelerates the development and spread of resistance. Furthermore, antimicrobial resistance surveillance systems remain insufficient in many parts of the country. Generating institution-specific and region-specific AMR data is therefore essential to support clinical management, guide antimicrobial stewardship programs, and inform national AMR containment strategies. Therefore, this study aimed to determine the prevalence and antimicrobial resistance patterns of Gram-negative bacterial isolates tested from 2018 to 2024 at the Amhara Public Health Institute, Bahir Dar, Northwest Ethiopia.

MATERIALS AND METHODS

Study setting, design and period

An institution-based retrospective cross-sectional study was conducted at the Amhara Public Health Institute (APHI), located in Bahir Dar, Amhara National Regional State, Northwest Ethiopia. The Amhara Public Health Institute serves as a regional reference laboratory, receiving clinical specimens from hospitals and health facilities across Bahir Dar and surrounding areas. The bacteriology reference laboratory performs bacterial identification and antimicrobial susceptibility testing following standard microbiological procedures. The study analyzed archived microbiology laboratory data of Gram-negative bacterial isolates obtained from clinical specimens collected between January 2018 and December 2024.

Study population and sample size

The study included all clinical specimens that yielded Gram-negative bacterial isolates and had complete antimicrobial susceptibility testing (AST) results during the study period. A census sampling method was used, and all eligible laboratory records meeting the inclusion criteria were included in the analysis.

Inclusion and Exclusion Criteria

Laboratory-confirmed Gram-negative bacterial isolates with

https://doi.org/10.20372/ejhbs.1129

complete antimicrobial susceptibility testing results, and records within the study period (2018–2024) were included in this study. While those incomplete laboratory records and missing antimicrobial susceptibility results were excluded.

Data collection procedures

Socio-demographic and laboratory data were retrieved from archived laboratory records at the Microbiology Reference Laboratory of APHI using a structured data extraction format developed in Microsoft Excel. The extracted variables included age, sex, year of testing, type of clinical specimen, bacterial isolates, and antimicrobial susceptibility results.

Clinical specimens used for isolation of the Gram-negative bacteria were urine, blood, sputum, wound/pus, cerebrospinal fluid, body fluids, ear discharge, eye discharge, throat swabs, and other relevant specimen types. To ensure confidentiality, a unique identification code was assigned to each patient record, and no personal identifiers were used.

Bacterial identification and antimicrobial susceptibility test

Clinical specimens were processed using the routine standard microbiological techniques at the Amhara Public Health Institute. Specimens were inoculated onto appropriate culture media and incubated at 37 °C. Bacterial isolates were identified based on colony morphology, Gram reaction, and biochemical tests.

Antimicrobial susceptibility test was performed using the Kirby–Bauer disk diffusion method on Mueller-Hinton agar, following the Clinical and Laboratory Standards Institute (CLSI M100) guidelines. Bacterial suspensions were prepared and adjusted to 0.5 McFarland turbidity standard and inoculated onto Mueller–Hinton agar plates. Antibiotic disks were applied, and plates were incubated at 37 °C for 16–18 hours. The diameter of inhibition zones was measured and interpreted as susceptible, intermediate, or resistant according to the CLSI criteria.

The following antibiotics were tested: Amoxicillin/clavulanic acid (20/10 µg), Ampicillin (10 µg), Cefepime (30 µg), Cefotaxime (30 µg), Cefoxitin (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Ciprofloxacin (5 µg), Chloramphenicol (30 µg), Gentamicin (10 µg), Tobramycin (10 µg), Trimethoprim–sulfamethoxazole (1.25/23.75 µg), Nitrofurantoin (300 µg), Imipenem (10 µg), Meropenem (10 µg), and Tetracycline (15 µg).

Multidrug resistance (MDR) was defined as resistance to at least one antibiotic agent in three or more antimicrobial classes.

Data management and Analysis

Data were checked for completeness, coded, and entered into Epi Info version 7.2.5, and then exported to SPSS version 26.0 for analysis. Descriptive statistics, including frequencies, percentages, and proportions, were used to summarize bacterial prevalence and antimicrobial resistance patterns. The prevalence of multidrug resistance was calculated as the proportion of isolates resistant to at least one antibiotic in three or more antimicrobial classes. The Chi-square test was used to assess associations between bacterial isolates and demographic variables such as age and sex of patients. A p-value of less than 0.05 was considered statistically significant.

Ethical considerations

Ethical approval was obtained from the Institutional Review Board (IRB) of the Amhara Public Health Institute (Protocol No: IRB NoH/R/T/T/D/07/74). Permission was also obtained from the APHI laboratory diagnostic directorate.

The requirement for informed consent was waived due to the retrospective nature of the study. All data were anonymized, and confidentiality was maintained throughout the study. The study was conducted in accordance with the Declaration of Helsinki.

RESULTS

Prevalence of Gram-negative bacterial isolates

A total of 350 Gram-negative bacterial isolates were identified from the different clinical specimens processed. Overall, *Escherichia coli* was the most frequently isolated organism, accounting for 29.4% (103/350; 95% CI: 24.6–34.2) of all isolates. This was followed by *Klebsiella* spp., which comprised 27.7% (97/350; 95% CI: 23.0–32.4). *Acinetobacter baumannii* and *Enterobacter* spp. accounted for 12.6% (44/350; 95% CI: 9.1–16.1) and 12.0% (42/350; 95% CI: 8.6 –15.4) of isolates, respectively.

Pseudomonas aeruginosa represented 10.0% (35/350; 95% CI: 6.9–13.1) of the total isolates. Lower prevalence was observed for *Proteus* spp., accounting for 5.1% (18/350; 95% CI: 2.8–7.4), and *Citrobacter* spp., which comprised 3.1% (11/350; 95% CI: 1.3–5.0) of isolates (**Table 1**).

Table 1: Proportion of Gram-negative bacteria isolated at Amhara Public Health Institute, 2018 -2024

Bacterial isolates	Frequency	Percentage	95% CI
A. baumannii	44	12.6	9.1 – 16.1
Citrobacter spp.	11	3.1	1.3 – 5.0
E. coli	103	29.4	24.6 – 34.2
Enterobacter spp.	42	12.0	8.6 – 15.4
Klebsiella spp.	97	27.7	23.0 – 32.4
Proteus spp.	18	5.1	2.8 – 7.4
P. aeruginosa	35	10.0	6.9 – 13.1

Distribution of the Gram-negative bacterial isolates by age group and sex

The distribution of Gram-negative bacterial isolates varied significantly across different age groups (χ^2 = 106.69, p < 0.001). *Klebsiella* spp. and *Enterobacter* spp. were more frequently isolated among children under five years of age, accounting for 52.6% and 35.7% of isolates in this age group, respectively. *Acinetobacter baumannii* was most common among individuals aged 15–20 years (27.3%). *Proteus* spp. and *Pseudomonas aeruginosa* were predominantly isolated from patients aged 20–50 years, representing 72.2% and 65.7% of isolates in this age group, respectively. In contrast, *Escherichia coli* was more frequently isolated among patients aged above 50 years, accounting for 32.0% of isolates in this age category.

The distribution of Gram-negative bacterial isolates also showed a statistically significant association with sex (χ^2 = 12.86, p = 0.045). *Escherichia coli* was more commonly isolated among female patients, accounting for 56.3% of isolates. In contrast, *Proteus* spp. (77.8%), *Pseudomonas aeruginosa* (60.0%), and *Acinetobacter baumannii* (54.5%) were more frequently isolated from male patients (**Table 2**).

Table 2: Distribution of Gram-Negative bacterial isolates across age and sex of study participants at Amhara Public Health Institute, 2018 -2024

	Isolated organisms							
Variables	***A. baumannii* (n=44)**	***Citrobacter* spp. (n=11)**	***E. coli* (n=103)**	***Enterobacter* spp. (n=42)**	***Klebsiella* spp. (n=97)**	***Proteus* spp. (n=18)**	***P. aeruginosa* (n=35)**	**χ^2 (p-value)**
Age in years								89.6 (< 0.001)
< 5	18 (40.9%)	1 (9.1%)	15(14.6%)	23 (54.8%)	68 (70.1%)	1 (5.6%)	7 (20.0%)	
> 50	9 (20.5%)	2 (18.2%)	26(25.2%)	2 (4.8%)	2 (2.1%)	2 (11.1%)	3 (8.6%)	
20 – 50	12 (27.3%)	6 (54.5%)	51(49.5%)	12(28.6%)	17(17.5%)	12(66.7%)	18(51.4%)	
5 to 20	5(11.4%)	2(18.2%)	11(10.7%)	5(11.9%)	10(10.3%)	3(16.7%)	7(20.0%)	
Sex								14.8 (0.022)
Female	17(38.6%)	6(54.5%)	53(51.5%)	17(40.5%0	36(37.1%)	3(16.7%)	10(28.6%)	
Male	27(61.4%)	5(45.5%)	50(48.5%)	25(59.5%)	61(62.9%)	15(83.3%)	25(71.4%)	

Antimicrobial resistance patterns of Gram-negative bacteria

The antimicrobial resistance patterns varied across antibiotic classes. Overall, high resistance rates were observed against commonly used antibiotics. The highest resistance rates were observed against Chloramphenicol (97.3%), Ampicillin (82.4%), and Sulfonamides (80.3%), Cephalosporins (48.6–67.9%), Fluoroquinolones (42.5%), and Tetracycline (36.2%). Relatively, lower resistance was observed for Aminoglycosides (30.0%) and Carbapenems (24.9%) (**Table 3**).

https://doi.org/10.20372/ejhbs.1129

Table 3: Multi drug resistance of Gram-negative bacteria isolated at Amhara Public Health Institute, 2018 -2024

Antibiotic Class	**Antibiotics**	***A. baumannii***	***Citrobacter Spp.***	***E. aerogenes***	***E. Coli***	***Proteus spp.***	***Pseudomonas Spp.***	***Klebsiella Spp.***	**Total**
Penicillin	Amp	66.7%	81. %8	80%	70.8%	100%	100%	76.9%	82.4%
	AMC	83.3%	54. %5	100%	66.7%	31.5%	75%	84.6%	79.4%
Phenicol	CH	100 %	70%	100%	91.7%	100%	100%	92.3%	97.3%
Aminoglycosides	TOB	50 %	54.5%	20%	33.3%	54.6%	25%	69.2%	32.9%
	GN	50 %	54.4%	20%	20.8%	35.4%	25%	69.2%	26.7%
Cephalosporin	CTR	50 %	63.6%	40%	41.7%	53.1%	50%	69.2%	47.4%
	Cefta	50 %	72.7%	20%	45.8%	92.3%	50%	61.5%	37.9%
	Cefox	33.3%	68%	20%	29.2%	72%	50%	30.8%	32.8%
Fluoroquinolones	CIP	33.3 %	54.5%	100%	62.5%	40.7%	100%	76.9%	73.2%
Sulfonamides	CoT	100 %	72.7%	100%	70.8%	78.5%	75%	69.2%	80.3%
Nitrofurans	F	100 %	76%	20%	33.3%	68%	86%	84.6%	39.7%
Tetracycline	TE	66.7%	72.7%	60%	54.2%	76.9%	70%	56%	36.2%
Carbapenem	IMP	83.3%	63.6%	20%	45.8%	21.6%	20%	21%	24.9%

Amp: Ampicillin, AMC: Amoxicillin-clavulanic acid, CH: Chloramphenicol, TOB: Tobramycin, GN: Gentamicin. CTR: Ceftriaxone, Cefta: Ceftazidime, Cefox: Cefotaxime, CIP: Ciprofloxacin, CoT: Cotrimoxazole, F: *Nitrofurantoin*, TE: tetracycline, IMP: Imipenem.

DISCUSSION

The present study assessed the prevalence and antimicrobial resistance patterns of Gram-negative bacteria isolated from clinical specimens collected over a six-year period (2018–2024) at the Amhara Public Health Institute, Northwest Ethiopia. In this study, *Escherichia coli* was the most frequently isolated organism, accounting for 29.4% (95% CI: 24.6–34.2) of all isolates, followed by *Klebsiella* spp. (27.7%, 95% CI: 23.0–32.4). This finding is consistent with previous studies conducted in Ethiopia, which reported *E. coli* and *Klebsiella* spp. as the most predominant Gram-negative pathogens (10–13). The slight variation in proportions across studies may be attributed to differences in study population, specimen types, healthcare settings, and sample size.

Age-specific distribution showed that *Klebsiella* spp. and *Enterobacter* spp., followed by *Acinetobacter baumannii*, were more prevalent among children under five years of age. This finding aligns with previous reports indicating *Klebsiella pneumoniae* as one of the leading multidrug-resistant pathogens among pediatric populations (14). Among adults, *Proteus* spp. were the most frequently isolated organisms, which is comparable to findings from India reporting higher prevalence of *Proteus* species among adult populations, particularly in middle-aged and older individuals (15).

Sex-based analysis revealed that *E. coli* was the most prevalent organism among females, followed by *Klebsiella* spp., whereas *Proteus* spp. were the predominant isolates among males. This finding is consistent with a study conducted in Brazil that reported higher prevalence of *E. coli* among females and *Proteus mirabilis* among males (16). These differences may reflect variations in anatomical, physiological, and behavioral risk factors influencing infection susceptibility.

The present study also revealed a substantial burden of multidrug resistance among Gram-negative bacteria, with an overall MDR prevalence of 53.2%. This indicates that more than half of the isolates were resistant to at least three classes of antibiotics, highlighting the growing challenge of antimicrobial resistance in the study setting. This finding is consistent with a systematic review conducted in Ethiopia, which reported MDR prevalence ranging from 30% to 85% among bacterial pathogens (17). These findings emphasize the increasing limitations of empirical antibiotic therapy and underscore the importance of routine antimicrobial susceptibility testing. Furthermore, these results align with global evidence indicating that Africa bears a disproportionate burden of antimicrobial resistance due to limited diagnostic capacity, unregulated antibiotic use, and resource constraints (5).

Analysis of antibiotic resistance patterns showed that Gram-negative bacteria exhibited high resistance to commonly used antibiotics. The highest resistance rates were observed for phenicol (97.3%), sulfonamides (80.3%), and penicillin (81%). Resistance to specific antibiotics was particularly high for chloramphenicol (97.3%), ampicillin (82.4%), amoxicillin-clavulanate (79.4%), ciprofloxacin (73.2%), and ceftriaxone (47.4%). In contrast, relatively lower resistance rates were observed for carbapenems (24.9%), aminoglycosides (30%), tetracyclines (36.2%), and nitrofurantoin (39.7%). These findings are consistent with studies conducted in Ethiopia, Tanzania, and Rwanda, which reported high resistance to commonly used antibiotics and relatively preserved effectiveness of carbapenems and aminoglycosides (18–20).

Organism-specific analysis showed that *E. coli* exhibited high resistance to phenicol and sulfonamides, moderate resistance to penicillin, fluoroquinolones, and tetracyclines, and lower resistance to carbapenems and aminoglycosides. Similar resistance patterns have been reported in studies conducted in China and other regions (21). *Klebsiella* spp. demonstrated high resistance to phenicol, fluoroquinolones, and penicillin, moderate resistance to cephalosporins and sulfonamides, and relatively low resistance to carbapenems. These findings are consistent with previous reports from Ethiopia (22).

A. baumannii exhibited extremely high resistance to multiple antibiotic classes, including complete resistance to phenicol, sulfonamides, and nitrofurantoin, and high resistance to carbapenems. These findings are consistent with studies conducted in Ethiopia, Nigeria, and Saudi Arabia, which reported widespread multidrug resistance among *Acinetobacter* species (23–26).

Similarly, *Citrobacter* spp. showed high resistance to sulfonamides, nitrofurans, and tetracyclines, with moderate resistance to penicillin, cephalosporins, and fluoroquinolones. These findings are supported by studies conducted in Ethiopia, Bangladesh, and systematic reviews reporting high resistance among *Citrobacter* isolates (27–29). *Enterobacter aerogenes* demonstrated extremely high resistance to phenicol, fluoroquinolones, and sulfonamides, but relatively low resistance to carbapenems and aminoglycosides, consistent with previous findings (30).

Proteus spp. exhibited high resistance to phenicol, cephalosporins, and sulfonamides, while maintaining relatively lower resistance to carbapenems and aminoglycosides. These findings align with studies conducted in Egypt and other regions (31). Likewise, *Pseudomonas* spp. showed high resistance to phenicol, fluoroquinolones, and penicillin, with lower resistance to carbapenems and aminoglycosides, consistent with reports from Ethiopia and Iraq (32). Overall, the high prevalence of multidrug resistance and widespread resistance to commonly used antibiotics observed in this study highlight the growing challenge of antimicrobial resistance in the study setting. The relatively lower resistance observed for carbapenems and aminoglycosides suggests that these antibiotics remain important treatment options; however, their use should be carefully monitored to prevent further resistance development.

Limitations of the study

This study has several limitations. First, due to its retrospective design, important clinical variables such as prior antibiotic use, hospitalization history, comorbidities, clinical outcomes, and infection source were not available. Second, the study was conducted at a single public health institute, which may limit the generalizability of the findings to other regions of Ethiopia.

CONCLUSION AND RECOMMENDATION

This study demonstrated a high prevalence of multidrug-resistant Gram-negative bacteria among clinical isolates. The most frequently isolated organisms were *Escherichia coli*, *Klebsiella* spp., and *Acinetobacter baumannii*. More than half of the isolates were multidrug resistant, with high resistance observed against commonly used antibiotics such as chloramphenicol, sulfonamides, and penicillin. In contrast,

https://doi.org/10.20372/ejhbs.1129

the isolates showed relatively lower resistance to carbapenems and aminoglycosides.

These findings highlight the urgent need to strengthen antimicrobial resistance surveillance, promote routine culture and susceptibility testing, and implement antimicrobial stewardship programs.

Authors' contributions

AA conceived and designed the study, performed the data analysis, and drafted the manuscript. MG, GB, and DS contributed to data analysis, interpretation of the findings, and critical revision of the manuscript. All authors read and approved the final version of the manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

Declarations

Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

Availability of Data and Materials

All relevant data generated or analyzed during this study are included in this published article. The original dataset is available from the corresponding author upon reasonable request.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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