Nepal J Biotechnol. 2025 Dec; 13 (2): 111-117 **Research Article** DOI: https://doi.org/10.54796/njb.v13i2.353

Nepal Journal of Biotechnology
Publisher: Biotechnology Society of Nepal ISSN (Online): 2467-9313
Journal Homepage: https://nepjb.com/index.php/NJB ISSN (Print): 2091-1130

Antibiotic Susceptibility Profile of Gram-Negative Bacteria Isolated from Clinical Samples in a Tertiary Care Hospital

Anima Adhikari, Shushant Dahal, Sebika Poudel Upadhyaya, Nikita Pudasaini, Rabina Gurung, Sarita Manandhar

Department of Microbiology, Tri-Chandra Multiple Campus, Tribhuvan University, Kathmandu, Nepal

Received: 14 Jan 2025; Revised: 12 Oct 2025; Accepted: 28 Nov 2025; Published online: 31 Dec 2025

Abstract

The increasing occurrence of antimicrobial resistance in Gram-negative bacteria poses a significant challenge to healthcare systems worldwide. This study aimed to assess the antimicrobial susceptibility patterns of Gram-negative bacteria isolated from diverse clinical samples at Sukraraj Tropical and Infectious Disease Hospital in Teku, Kathmandu. A total of 737 clinical samples (blood, sputum, and urine) were collected over two months, April 2023 to June 2023 and processed for bacterial culture and identification. The Gram-negative bacteria were identified following conventional methods including Gram staining and various biochemical tests. An antibiotic susceptibility test was performed using the modified Kirby-Bauer disc diffusion method on Mueller Hinton agar (MHA) media. The isolates were tested for multi-drug resistance as well as Extended-spectrum beta-lactamase (ESBL). Of the 74 bacterial isolates, 62 (83.8%) were Gram-negative. The most prevalent bacteria were found to be *E. coli* (26, 41.9%) followed by *S.* Typhi (12, 19.4%), *Pseudomonas* spp. (9, 14.5%) and *Klebsiella* spp. (8, 12.9%). Considering the frequency of antibiotics used, isolates showed the highest sensitivity to gentamicin (54, 87.1%). In contrast, the highest resistance was observed against cefixime (24, 38.7%). Among the 26 *E. coli* isolates, 13 (50%) were detected as multidrug-resistant (MDR). Among the 30 urinary isolates examined, 12 (40%) tested ESBL positive during preliminary screening. Of these 12 preliminary positives, only one isolate was confirmed to produce ESBL. The findings emphasize the necessity of ongoing monitoring of antibiotic resistance patterns and the crucial role of antibiotic stewardship in controlling the spread of resistant Gram-negative bacteria in clinical environments.

Keywords: Gram-negative bacteria, antibiotic susceptibility, multidrug resistance, ESBL, clinical samples

Corresponding author, email: sarita.manandhar@trc.tu.edu.np

Introduction

Gram-negative bacteria exhibit a wide range of microorganisms that stand out due to their unique cell wall structure and staining behavior. This distinction is observed when applying the Gram stain technique, pioneered by Hans Christian Gram in the 1880s. These bacteria appear pink or red under a microscope after the staining process, which reflects their thinner peptidoglycan layer in the cell wall [1].

Gram-negative bacteria comprise many different species, many of which are dangerous and can cause a variety of diseases in humans, animals, and plants. Gram-negative pathogens include *Escherichia coli, Salmonella* spp., *Neisseria gonorrhoeae, Enterobacter* spp., *Pseudomonas* spp, *Moraxella* spp., *Helicobacter* spp., *Klebsiella pneumoniae*, to name a few. Gram-negative bacteria have a characteristic cell wall structure that consists of an outer membrane made of lipopolysaccharides (LPS), phospholipids, and proteins that act as a barrier to many drugs and host immune systems [2]. The periplasmic space, which exists between the outer and inner membranes, contains a variety of enzymes and proteins that play critical roles in food uptake, cell wall remodeling, and tolerance to external stresses [3, 4].

Gram-negative bacteria cause a range of diseases, with notable examples including *E. coli* causing gastrointestinal and urinary tract infections, *Salmonella* leading to food poisoning, and enteric fever, and *K. pneumoniae* causing hospital-acquired pneumonia and urinary tract infections [5, 6, 7].

Antibiotic resistance underscores the need for cautious treatment approaches against these Gram-negative bacteria. Infections caused by multidrug-resistant (MDR) bacteria have become a major concern in recent years [8]. MDR is characterized by bacteria's ability to withstand and reproduce even in the presence of multiple antibiotics frequently employed to treat infections caused by Gram-negative bacteria [9]. This resistance arises due to the overuse and misuse of antibiotics, which allows bacteria to develop mechanisms to counteract the drugs. Microorganisms utilize various mechanisms to develop drug resistance, including horizontal gene transfer via plasmids, transposons, and bacteriophages, integration of foreign DNA into bacterial chromosomes through recombination, and mutations in different chromosomal loci. These adaptive processes contribute to the growing

challenge of treating infections caused by resistant bacterial strains [10, 11]. Antimicrobial resistance (AMR) issues can be exacerbated in developing countries like Nepal due to the availability and misuse of antibiotics without proper medical authorization or supervision [12]. Unrestricted access to antibiotics can lead to their inappropriate use, including overuse, under-dosing, or using antibiotics for non-bacterial infections, which contributes to the development of drug-resistant bacterial strains [13]. This highlights the significance of implementing effective regulations and awareness programs to ensure responsible antibiotic use and combat the rising challenge of antimicrobial resistance in developing nations [14, 15].

MDR poses a substantial challenge in the healthcare domain. Gaining insights into the mechanisms behind MDR in different infections caused by Gram-negative bacteria is vital for developing effective treatment and prevention strategies. The inappropriate use of antibiotics can lead to the development of resistance mechanisms in bacteria, enabling them to survive and proliferate despite antibiotic exposure [16, 17].

One such mechanism involves the production of extended-spectrum beta-lactamase (ESBLs), which are enzymes capable of inactivating beta-lactam antibiotics, rendering them ineffective. Additionally, some bacteria produce efflux pumps, which serve to expel antibiotics from within the cell, thereby preventing their accumulation and rendering the treatment less effective. Understanding these resistance mechanisms and their implications is essential in the battle against MDR in infections and reinforces the importance of responsible antibiotic usage to preserve the efficacy of these critical medications. The increasing use of broad-spectrum cephalosporin antibiotics has become a major factor in the rising prevalence of ESBL-producing microorganisms. The overuse and misuse of these powerful antibiotics can exert selective pressure on bacteria, favoring the survival and proliferation of ESBL-producing strains. Consequently, the prevalence of ESBL-producing microorganisms rises, making infections caused by such bacteria increasingly difficult to treat due to their resistance to multiple antibiotics [18, 19, 20].

Materials and methods

Sample collection and processing

This was a descriptive cross-sectional study conducted at the Department of Microbiology, Sukraraj Tropical and Infectious Disease Hospital, Teku, Kathmandu. A total of 737 blood, sputum, and urine samples from patients visiting the hospital were studied over the duration of two months from April 2023 to June 2023.

Urine, blood, and sputum samples were collected under aseptic conditions. Midstream urine specimens were collected in sterile, wide-mouthed containers. Blood samples were obtained by venipuncture using sterile vacutainers. Sputum samples were collected in sterile, leak-proof containers after instructing patients to provide an early-morning deep cough specimen. All specimens were labeled, transported to the laboratory and processed within 2–4 hours of collection.

A calibrated loop (0.001 ml) was used to inoculate urine samples onto Cystine Lactose Electrolyte-Deficient (CLED) agar, MacConkey agar (MA) and Blood agar (BA), and incubated aerobically at 37°C for 18–24 hours. Growth of ≥10^5 CFU/ml was considered significant for bacteriuria.

Blood sample (5-10 ml) were inoculated aseptically into BACTEC culture bottles. Bottles were loaded into the BACTEC automated blood culture system and continuously monitored for microbial growth. When flagged positive by the instrument, samples were sub cultured onto BA and MA. Plates were incubated at 37°C for 24–48 hours to obtain isolated colonies.

Sputum samples were directly inoculated using sterile loops onto BA, MA and Chocolate agar (CA). MA and BA plates were incubated at 37°C for 24–48 hours in aerobic conditions while CA in 5–10% CO_2.

Isolation and Identification of Gram-negative bacteria

The bacterial isolates were identified through standard microbiological methods, including colony morphology on different culture media, Gram staining, and a series of biochemical tests [21].

Antibiotic susceptibility test

The Kirby-Bauer disc diffusion method was used to assess the antibiotic susceptibility of the isolates. Briefly, a sterile cotton swab was used to inoculate the MHA plate with a bacterial suspension adjusted to the 0.5 McFarland standard using the lawn culture method. Using sterile forceps, various antibacterial discs such as cefixime at (5 µg), ciprofloxacin (5 µg), cotrimoxazole (25 µg), ceftriaxone (30 µg), gentamicin (10 µg), and nitrofurantoin (300 µg) were placed with even distribution. The plate was then incubated at 37°C for 24 hours. Following incubation, the zone of inhibition was measured to determine whether the bacteria were resistant or sensitive to the antibiotic. An organism was classified as multidrug-resistant (MDR) if it exhibited

resistance to at least one agent in three or more antibiotic classes [22, 23].

Detection of ESBL producer

Only *E. coli* isolates were tested for their ability to produce ESBL. The preliminary test for potential ESBL producers was done using the antibiotics ceftazidime and cefotaxime. The zone of inhibition around the antibiotics was measured. For any isolates with zone of inhibition ≤ 22 mm around ceftazidime and ≤ 27 mm around cefotaxime, they are considered resistant to the antibiotics and potential ESBL producers.

For phenotypic confirmation of ESBL production, plates containing cefotaxime (30 µg) or ceftazidime (30 µg) combined with clavulanate (10 µg) were used, following CLSI guidelines [24]. A lawn culture of *E. coli* was prepared on an MHA plate, and the antibiotic discs were placed 15 mm apart before being incubated aerobically overnight at 37°C. ESBL production was indicated by a ≥5 mm increase in the zone of inhibition around the cephalosporin discs compared to their clavulanate-containing counterparts [25, 26, 27]

Data Compilation and Statistical Analysis

The data were entered into Microsoft Excel 2013 and statistical studies utilized SPSS statistical analysis software (SPSS, Inc., Chicago, IL, USA) version 22. The significance of the obtained results was judged at the 0.05 level. The Chi-square test was used for comparing two or more groups.

Results

Growth pattern among the processed samples

Out of 737 sample processed, 263 (35.7%) were urine samples, 260 (35.3%) were sputum samples and 214 (29.1%) were blood samples. Bacterial growth was observed in 74 (10.05%) samples, while growth was not detected in the remaining 663 (89.95%) samples (**Figure 1**).

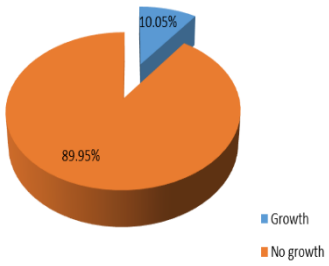

Figure 1. Pie chart showing the percentage of growth among processed samples

Demographic distribution of samples

Bacterial growth was most frequently observed in samples from the 40–59 age group (24, 32.4%). Growth was more common in male patients (50, 67.6%) than in females (24, 32.4%). In addition, most of the samples from the outpatients (69, 93.2%) showed growth. Among clinical specimens, urine showed the highest growth (32, 43.2%). Although age appeared to influence the distribution of bacterial isolates, no significant association was found with gender, hospital ward or type of clinical specimen (**Table 1**).

Table 1. Demographic distribution of samples

Aspects	Growth (N=74)		No growth (N=663)		Total (N=737)		P-Value
	n	%	n	%	n	%	
Age group							
≤19	8	10.8	54	8.1	62	8.4	
20-39	20	27.0	300	45.2	320	43.4	
40-59	24	32.4	196	29.6	220	29.9	0.0177
60-79	17	23.0	95	14.3	112	15.2	
80-99	5	6.8	18	2.7	23	3.1	
Gender							
Male	50	67.6	379	57.2	429	58.2	0.9393
Female	24	32.4	284	42.8	308	41.8	
Ward							
Out patients	69	93.2	633	95.5	702	95.3	0.3904
In patients	5	6.8	30	4.5	35	4.7	
Clinical Specimen							
Urine	32	43.2	231	34.8	263	35.7	
Blood	19	25.7	195	29.4	214	29.0	0.3110
Sputum	23	31.1	237	35.7	260	35.3	

Bacteriological profile of Gram-negative and Gram-positive isolates

Among the 74 positive cases, Gram-negative bacteria were identified in 62 (83.8%), while Gram-positive bacteria were found in 12 (16.2%) cases. Among all bacteria identified, *E. coli* exhibited the highest prevalence at 26 (35.1%). *Salmonella* Typhi and *Klebsiella* spp. were reported to have a prevalence rate of 12 (16.2%) and 8 (10.8%) respectively. Additionally, 4 (80.0%) samples were from inpatients and 58 (84.1%) were from outpatients (**Table 2**).

Among the Gram-positive isolates, *Staphylococcus aureus* displayed the highest prevalence both in blood and sputum samples i.e. 33.3%. In addition, out of 12 samples showing growth, only one was from an inpatient.

Table 2. Bacteriological profile of Gram-negative isolates

Sample	Isolates	Inpatient (N=5)		Outpatient (N=69)		Total (N=74)	
		n	%	n	%	n	%
Urine	*Escherichia coli*	1	20.0	24	34.8	25	33.8
	Proteus mirabilis	-	-	2	2.9	2	2.7
	Citrobacter spp.	-	-	3	4.3	3	4.1
Blood	*Escherichia coli*	-	-	1	1.4	1	1.4
	Salmonella Typhi	-	-	12	17.4	12	16.2
Sputum	*Klebsiella* spp.	1	20.0	7	10.1	8	10.8
	Acinetobacter spp.	-	-	1	1.4	1	1.4
	Pseudomonas spp.	2	40.0	7	10.1	9	12.2
	Enterobacter spp.	-	-	1	1.4	1	1.4
Total		4	80.0	58	84.1	62	83.8

Antibiotic susceptibility pattern of urinary isolates

Gentamicin and nitrofurantoin were the most effective antibiotics against all bacterial isolates, showing a high sensitivity rate (27, 90%) each. In contrast, most of the isolated bacteria were resistant to cotrimoxazole (18, 60%) (**Table 3**).

Table 3. Antibiotic susceptibility profile of urinary isolates (N=30)

Antibiotics used	Resistant		Sensitive	
	n	%	n	%
Cefixime	16	53.3	14	46.7
Ceftriaxone	14	46.7	16	53.3
Ciprofloxacin	15	50	15	50
Gentamicin	3	10	27	90
Nitrofurantoin	3	10	27	90
Cotrimoxazole	18	60	12	40

Antibiotic susceptibility pattern of sputum isolates

Ciprofloxacin and gentamicin demonstrated the highest sensitivity (16, 84.2%). Conversely, the isolates exhibited the highest resistance to cefixime (7, 36.8%) (**Table 4**).

Table 4. Antibiotic susceptibility profile of sputum isolates (N=19)

Antibiotics used	Resistant		Sensitive	
	n	%	n	%
Cefixime	7	36.8	12	63.2
Ceftriaxone	5	26.3	14	73.6
Ciprofloxacin	3	15.8	16	84.2
Gentamicin	3	15.8	16	84.2
Cotrimoxazole	5	26.3	14	73.6

Antibiotic susceptibility pattern of blood isolates

Cotrimoxazole was the most effective antibiotic against all tested isolates (13, 100%), while ciprofloxacin exhibited the highest resistance rate (3, 23%) (**Table 5**).

Table 5. Antibiotic susceptibility profile of blood isolates (N=13)

Antibiotics used	Resistant		Sensitive	
	n	%	n	%
Cefixime	1	7.7%	12	92.3%
Ceftriaxone	1	7.7%	12	92.3%
Ciprofloxacin	3	23%	10	77%
Gentamicin	2	15.4%	11	84.6%
Cotrimoxazole	-	-	13	100%

Susceptibility profile of Gram-negative isolates to antibiotics

Nitrofurantoin exhibited the highest sensitivity rate against Gram-negative isolates (27, 90%), while gentamicin showed the greatest number of susceptible isolates (54, 87.1%). Conversely, marked resistance was observed with cefixime (24, 38.7%) and cotrimoxazole (23, 37.1%) (**Table 6**).

Table 6. Antibiotic susceptibility profile of Gram-negative isolates (N=62)

Antibiotics used	Resistant		Sensitive	
	N	%	N	%
Cefixime	24	38.7%	38	61.3%
Ceftriaxone	20	32.3%	42	67.7%
Ciprofloxacin	21	33.9%	41	66.1%
Gentamicin	8	12.9%	54	87.1%
Nitrofurantoin	3	10%	27	90%
Cotrimoxazole	23	37.1%	39	62.9%

Multi-drug resistance (MDR) pattern of Gram-negative isolates

Among the 26 *E. coli* isolates, 13 (50%) were identified as multidrug-resistant. Of these, 5 (19.2%) isolates were resistant to two antibiotic classes, 3 (11.5%) isolates were resistant to three classes, another 3 (11.5%) isolates were resistant to four classes, and 2 (7.7%) isolates were resistant to five distinct antibiotic classes. Additionally,

out of the 8 *Klebsiella spp.* isolates, only 3 (37.5%) were multi-drug resistant. Of these, 2 (25%) isolates were resistant to three different classes, and 1 (12.5%) isolate was resistant to five classes (Figure 2).

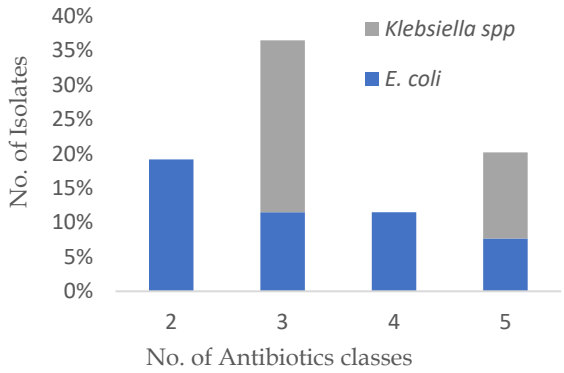

Figure 2. MDR pattern of Gram-negative isolates

Urinary isolates producing extended-spectrum beta-lactamase (ESBL)

The preliminary test for potential ESBL producers was done using the antibiotics ceftazidime and cefotaxime. Among 30 urinary isolates, 12 displayed resistances towards either ceftazidime or cefotaxime, most of them being the isolates of *E. coli.* Plates with cefotaxime (30 µg) or ceftazidime (30 µg) combined with clavulanate (10 µg) were utilized for phenotypic confirmation of ESBL production. Out of 12 isolates tested positive during preliminary confirmation, only one isolate of *E. coli* (8.3%) was found to produce ESBL during the confirmatory test. (**Figure 3**).

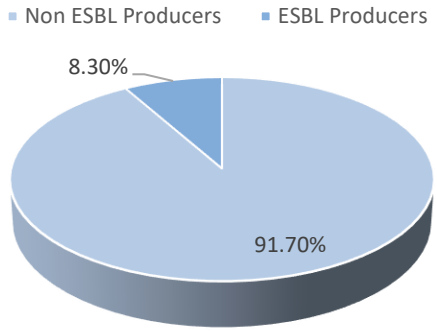

Figure 3. ESBL-producing *E. coli* isolates

Discussion

The current study showed the highest prevalence of Gram-negative bacteria (62, 83.78%) over Gram-positive bacteria (12, 16.22%). The high frequency of Gram-negative isolates reported in this study is consistent with global trends, highlighting their clinical importance. *E. coli* (25, 40.3%) was the most common followed by *Salmonella Typhi* (12, 19.4%) and *Klebsiella* spp. (7, 11.3%), whereas the lowest prevalence was found to be of *Enterobacter* spp. (1, 1.6%). A comparable study identified *E. coli* as the most prevalent bacterial isolate (179, 52.03%) [28]. The high prevalence of *E. coli* in clinical infections may be attributed to its ubiquitous presence in various environments, including natural habitats such as soil and water, as well as its colonization of the human body, particularly on the skin and within the intestinal tract. This widespread distribution enables *E. coli* to frequently come into contact with potential hosts, thereby increasing the likelihood of infection and its subsequent detection in clinical settings [29, 30].

Among the commonly used antibiotics for urinary isolates, gentamicin and nitrofurantoin were the most effective, each showing a sensitivity rate of 27 (90%). In contrast, most bacterial isolates showed resistance to cotrimoxazole and cefixime, with maximum resistance rates of 18 (60%) and 16 (53.3%), respectively. Our study supported the fact that *E. coli* has the highest prevalence rate among patients with urinary infections. A similar study showed that most of the urinary isolates showed 100% resistance to ampicillin while all UTI isolates exhibited least resistance against drugs such as ceftriaxone, gentamycin and chloramphenicol [31]. Another study found *E. coli* to be the predominant isolate, showing 100% susceptibility to intravenous antibiotics like carbapenems (imipenem and meropenem), 98.9% to amikacin, 96.5% to piperacillin-tazobactam, and 94.3% to gentamicin. Among oral antibiotics, the highest susceptibility rates were observed for fosfomycin (95.5%), nitrofurantoin (85.5%), and cefuroxime (82.3%) [32].

Sputum isolates showed high sensitivity to both ciprofloxacin and gentamicin (16, 84.2% each), while the highest resistance was observed against cefixime (7, 36.8%). A study conducted at Khadir Mohideen College in Tamil Nadu, India, revealed that the majority of sputum isolates were susceptible to amikacin, cefotaxime, ofloxacin, netilmicin, ciprofloxacin, and ceftriaxone [33].

Blood isolates in this study showed complete sensitivity to cotrimoxazole (13, 100%) and high sensitivity to amoxicillin, cefixime, and ceftriaxone (12, 92.3% each), while the highest resistance was noted against ciprofloxacin (3, 23%). These results differ from an Ethiopian study, where Gram-negative isolates exhibited high resistance to cotrimoxazole (53.1%), amoxicillin (58.8%), ampicillin (70.4%), tetracycline (75.9%), and gentamicin (76.9%). The discrepancy may be attributed to variations in local prescribing practices, antibiotic

availability, and infection control measures, highlighting the importance of region-specific surveillance. [34].

Antibiotic profiles of Gram-negative isolates provide beneficial information on commonly used antibiotics. The Gram-negative isolates exhibited higher susceptibility toward gentamicin, a broad-spectrum aminoglycoside, with a sensitivity rate of 87.1%. Nitrofurantoin is a nitrofuran derivative against which more than 90% of the urinary isolates such as *E. coli*, demonstrated sensitivity [35]. The most common cause of UTIs, *E. coli*, is very sensitive towards this antibiotic. This is reassuring with regard to the high sensitivity of nitrofurantoin, which is a relatively cheap and well-tolerated drug; thus, it can be considered a first-line drug in cases of uncomplicated UTI [36]. The rates of high resistance seen for cotrimoxazole and cefixime in Gram-negative isolates are 37.1% and 38.7%, respectively. Generally, cotrimoxazole is commonly used for treating UTIs and respiratory tract infections and the increasing resistance to this antibiotic is going to limit its utility [37]. Cefixime is a third-generation cephalosporin utilized in the management of infections caused by Gram-negative bacteria. The resistance rate in this study goes as high as 38.7%, which is a drawing concern since cephalosporins have relatively broad-spectrum properties and thus are considered the last resort drugs for MDR treatment. Overuse and misuse are the backgrounds for developing resistant strains of cephalosporins, such as those producing ESBLs [38].

The high prevalence of the MDR Gram-negative isolates, particularly *E. coli*, is a matter of considerable worry within the realm of public health, at 50% prevalence. MDR bacteria are resistant to multiple classes of antibiotics, reducing the number of options for the treatment of infections and thereby increasing the risk of treatment failure. This will lead most of the time to the emergence of MDR strains due to the abuse and misuse of drugs and poor infection control measures, especially in healthcare settings [39].

This study also examined the presence of ESBL-producing *E. coli* isolates in urinary samples. ESBL-type enzymes confer resistance to a broad range of beta-lactams, including third-generation cephalosporins. The ESBL production rate in this study was 8.3%. While this is a promising finding, it is important to highlight that ESBL-producing bacteria are linked to increased morbidity, mortality, and healthcare costs [40].

Conclusion

The study showed Gram-negative bacteria to be predominant in clinical samples, with *E. coli* being the most prevalent. Among Gram-negative isolates, concerning resistance patterns were observed, particularly for cotrimoxazole and cefixime. The high rates of MDR observed in *E. coli* and *Klebsiella* spp. are alarming, highlighting the need for continued monitoring of resistance patterns and formulation of evidence-based treatment guidelines based on local situations to limit the spread of resistant strains. Detection of ESBL-producing *E. coli* among urine samples further underscores the importance of routine ESBL screening in clinical settings. Thus, future research should focus on the mechanisms underlying antibiotic resistance in these pathogens and the development of strategies to mitigate the impact of MDR bacteria on public health. Implementing effective surveillance and promoting responsible antibiotic use are critical steps in addressing the challenges posed by antimicrobial resistance.

Author's Contributions

AA, SD, SPU, NP and RG performed experiment and prepared the manuscript. SM conceptualized the method, supervised the methods and edited the manuscript.

Competing interests

No competing interests were disclosed.

Funding

The authors declared that there were no grants involved in supporting this work.

Acknowledgements

The authors express their gratitude to the Department of Microbiology at Tri-Chandra Multiple Campus and Sukraraj Tropical & Infectious Disease Hospital for their invaluable support throughout the study period.

References

1. Tripathi N, Sapra A. Gram Staining. StatPearls - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK562156/
2. Maldonado RF, Sá-Correia I, Valvano MA. Lipopolysaccharide modification in Gram-negative bacteria during chronic infection. FEMS Microbiology Reviews. 2016 Apr 12;40(4):480–93. https://doi.org/10.1093/femsre/fuw007
3. Hirakawa H, Bordi C, Tomita H. Editorial: Gram-Negative pathogenesis. Frontiers in Microbiology. 2021 Dec 13;12. https://doi.org/10.3389/fmicb.2021.813062
4. Miller SI, Salama NR. The gram-negative bacterial periplasm: Size matters. PLoS Biology. 2018 Jan 17;16(1):e2004935. https://doi.org/10.1371/journal.pbio.2004935
5. Chang D, Sharma L, Cruz CSD, Zhang D. Clinical Epidemiology, Risk Factors, and Control Strategies of *Klebsiella pneumoniae* Infection. Frontiers in Microbiology. 2021 Dec 21;12. https://doi.org/10.3389/fmicb.2021.750662
6. Mueller M, Tainter CR. *Escherichia coli* Infection [Internet]. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2024 Jan–.

[Updated 2023 Jul 13]. https://www.ncbi.nlm.nih.gov/books/NBK564298/
7. Giannella RA. Salmonell. Medical Microbiology - NCBI Bookshelf. 1996. https://www.ncbi.nlm.nih.gov/books/NBK8435/
8. Bassetti M, Peghin M, Vena A, Giacobbe DR. Treatment of infections due to MDR Gram-Negative Bacteria. Frontiers in Medicine. 2019 Apr 16;6. https://doi.org/10.3389/fmed.2019.00074
9. Nikaido H. Multidrug resistance in bacteria. Annual Review of Biochemistry. 2009 Feb 20;78(1):119–46. https://doi.org/10.1146/annurev.biochem.78.082907.145923
10. Sun D, Jeannot K, Xiao Y, Knapp CW. Editorial: Horizontal Gene Transfer Mediated Bacterial Antibiotic Resistance. Frontiers in Microbiology. 2019 Aug 27;10. https://doi.org/10.3389/fmicb.2019.01933
11. Munita JM, Arias CA. Mechanisms of antibiotic resistance. Microbiology Spectrum. 2016 Mar 25;4(2). https://doi.org/10.1128/microbiolspec.vmbf-0016-2015
12. Ghimire K, Banjara MR, Marasini BP, Gyanwali P, Poudel S, Khatri E, et al. Antibiotics prescription, dispensing practices and antibiotic resistance pattern in common pathogens in Nepal: a Narrative review. Microbiology Insights. 2023 Jan 1;16:117863612311672. https://doi.org/10.1177/11786361231167239
13. Fleming-Dutra KE, Hersh AL, Shapiro DJ, Bartoces M, Enns EA, File TM, et al. Prevalence of inappropriate antibiotic prescriptions among US ambulatory care visits, 2010-2011. JAMA. 2016 May 3;315(17):1864. https://doi.org/10.1001/jama.2016.4151
14. Maharjan S, Gallagher P, Gautam M, Joh HS, Sujan MJ, Aboushady AT, et al. Recording and Reporting of Antimicrobial Resistance (AMR) priority Variables and its implication on expanding surveillance sites in Nepal: a CAPTURA experience. Clinical Infectious Diseases. 2023 Dec 15;77(Supplement_7):S560–8. https://doi.org/10.1093/cid/ciad581
15. Majumder MAA, Rahman S, Cohall D, Bharatha A, Singh K, Haque M, et al. Antimicrobial stewardship: Fighting antimicrobial resistance and protecting global public health. Infection and Drug Resistance. 2020 Dec 1; 13:4713–38. https://doi.org/10.2147/idr.s290835
16. Poole K. Multidrug resistance in Gram-negative bacteria. Current Opinion in Microbiology. 2001 Oct 1;4(5):500–8. https://doi.org/10.1016/s1369-5274(00)00242-3
17. Mehrad B, Clark NM, Zhanel GG, Lynch JP. Antimicrobial resistance in Hospital-Acquired Gram-Negative bacterial infections. CHEST Journal. 2015 May 1;147(5):1413–21. https://doi.org/10.1378/chest.14-2171
18. Paterson DL, Bonomo RA. Extended-Spectrum β-Lactamases: a Clinical Update. Clinical Microbiology Reviews. 2005 Oct 1;18(4):657–86. https://doi.org/10.1128/cmr.18.4.657-686.2005
19. Shilpakar A, Ansari M, Rai KR, Rai G, Rai SK. Prevalence of multidrug-resistant and extended-spectrum beta-lactamase producing Gram-negative isolates from clinical samples in a tertiary care hospital of Nepal. Tropical Medicine and Health. 2021 Mar 11;49(1). https://doi.org/10.1186/s41182-021-00313-3
20. Leylabadlo HE, Pourlak T, Bialvaei AZ, Aghzadeh M, Asgharzadeh M, Kafil HS. Extended-spectrum Beta-lactamase Producing Gram Negative Bacteria in Iran: A Review. African Journal of Infectious Diseases. 2017 Jun 8;11(2):39–53. https://pubmed.ncbi.nlm.nih.gov/28670639/
21. Mahon CR, Lehman DC. Textbook of Diagnostic Microbiology. Elsevier; 2022.
22. Karki D, Dhungel B, Bhandari S, Kunwar A, Joshi PR, Shrestha B, et al. Antibiotic resistance and detection of plasmid mediated colistin resistance mcr-1 gene among *Escherichia coli* and *Klebsiella pneumoniae* isolated from clinical samples. Gut Pathogens. 2021 Jul 5;13(1). https://doi.org/10.1186/s13099-021-00441-5
23. Gajic I, Kabic J, Kekic D, Jovicevic M, Milenkovic M, Culafic DM, et al. Antimicrobial susceptibility testing: A comprehensive review of currently used methods. Antibiotics. 2022 Mar 23;11(4):427. https://doi.org/10.3390/antibiotics11040427
24. CLSI. M100: Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. 2020. 104-105 p.
25. Health Protection Agency. Laboratory detection and reporting of bacteria with extended spectrum β-lactamases. National Standard Method QSOP 51 Issue 2.2. 2008 http://www.hpastandardmethods.org.uk/pdf_sops.asp.
26. Shakya, P., Shrestha, D. Drieux L, Brossier F, Sougakoff W, Jarlier V. Phenotypic detection of extended-spectrum β-lactamase production in Enterobacteriaceae: review and bench guide. Clinical Microbiology and Infection. 2008 Jan 1;14:90–103. https://doi.org/10.1111/j.1469-0691.2007.01846.x
27. Shakya P, Shrestha D, Maharjan E, Sharma VK, Paudyal R. ESBL Production Among *E. coli* and *Klebsiella spp*. Causing Urinary Tract Infection: A Hospital Based Study. The Open Microbiology Journal. 2017 Apr 28;11(1):23–30. https://doi.org/10.2174/1874285801711010023
28. Lamichhane B. Antibiotic Resistance Patterns of Gram Negative Isolates in a Tertiary Care Hospital of Nepal. Asian J Pharm Clin Res. 2014 Jul. 1;7(3):30-3. https://journals.innovareacademics.in/index.php/ajpcr/article/view/1112
29. Rubab M, Oh DH. Virulence Characteristics and Antibiotic Resistance Profiles of Shiga Toxin-Producing *Escherichia coli* Isolates from Diverse Sources. Antibiotics. 2020 Sep 8;9(9):587. https://doi.org/10.3390/antibiotics9090587
30. Geurtsen J, De Been M, Weerdenburg E, Zomer A, McNally A, Poolman J. Genomics and pathotypes of the many faces of *Escherichia coli*. FEMS Microbiology Reviews. 2022 Jun 24;46(6). https://doi.org/10.1093/femsre/fuac031
31. Beyene G, Tsegaye W. Bacterial uropathogens in urinary tract infection and antibiotic susceptibility pattern in Jimma University Specialized Hospital, Southwest Ethiopia. Ethiopian Journal of Health Sciences. 2011 Aug 26;21(2). https://doi.org/10.4314/ejhs.v21i2.69055
32. Jurałowicz E, Bartoszko-Tyczkowska A, Tyczkowska-Sieroń E, Kurnatowska I. Etiology and bacterial susceptibility to antibiotics in patients with recurrent lower urinary tract infections. Pol Arch Intern Med. 2020; 130: 373-381. https://www.mp.pl/paim/issue/article/15284/
33. Manikandan C, Amsath A. Antibiotic Susceptibility of Bacterial Strains Isolated from Patients with Respiratory Tract Infections. International Journal of Pure and Applied Zoology. 2013;1(1). https://www.alliedacademies.org/articles/antibiotic-susceptibility-of-bacterial-strains-isolatedfrom-patients-with-respiratory-tract-infections.pdf
34. Mulu W, Abera B, Yimer M, Hailu T, Ayele H, Abate D. Bacterial agents and antibiotic resistance profiles of infections from different sites that occurred among patients at Debre Markos Referral Hospital, Ethiopia: a cross-sectional study. BMC Research Notes. 2017 Jul 6;10(1). https://doi.org/10.1186/s13104-017-2584-y
35. Garau J. Other antimicrobials of interest in the era of extended-spectrum β-lactamases: fosfomycin, nitrofurantoin and tigecycline. Clinical Microbiology and Infection. 2008 Jan 1;14:198–202. https://doi.org/10.1111/j.1469-0691.2007.01852.x
36. Hooton TM. Uncomplicated urinary tract infection. New England Journal of Medicine. 2012 Mar 14;366(11):1028–37. https://doi.org/10.1056/nejmcp1104429
37. Rezaee M, Ghafourian S, Sayehmiri F, Pakzad R, Safiri S, Pakzad I. Prevalence of Cotrimoxazole Resistance Uropathogenic Bacteria in Iran: A Systematic Review and Meta-Analysis. Archives of Clinical Infectious Diseases. 2018 Aug 6;13(5). https://brieflands.com/articles/archcid-63256
38. Yousefipour M. Bacteria Producing Extended Spectrum β-lactamases (ESBLs) in Hospitalized Patients: Prevalence, Antimicrobial Resistance Pattern and its Main Determinants. Iranian Journal of Pathology. 2018 Dec 1;14(1):61–7. https://doi.org/10.30699/ijp.14.1.61
39. Bassetti M, Peghin M, Vena A, Giacobbe DR. Treatment of infections due to MDR Gram-Negative Bacteria. Frontiers in Medicine. 2019 Apr 16;6. https://doi.org/10.3389/fmed.2019.00074
40. Extended-spectrum beta-lactamase-producing bacteria isolated from hematologic patients in Manaus, State of Amazonas, Brazil. PubMed. 2011 Jul 1; https://doi.org/10.1590/S1517-838220110003000028