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Antimicrobial Susceptibility Profile of Gram-negative Bacteria Isolated from Clinical Samples in a Tertiary Care Hospital in Nepal

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Abstract

Infections caused by Gram-negative bacteria and the rapid emergence of antimicrobial resistance (AMR) represent a serious global public health challenge. Low and middle-income countries, including Nepal, face a significant burden of AMR due to irrational antibiotic use. This study aims to assess the antibiotic susceptibility patterns of Gram-negative bacteria isolated from a tertiary care hospital in Nepal. A hospital-based cross-sectional study was conducted at the Department of Microbiology, Sukraraj Tropical and Infectious Disease Hospital, Kathmandu, from April to July 2023. A total of 737 clinical specimens including urine (n=263), sputum (n=260) and blood (n=214) were processed using standard microbiological techniques. Antimicrobial susceptibility testing was performed using the modified Kirby–Bauer disk diffusion method following Clinical and Laboratory Standards Institute guidelines. Extended-spectrum β-lactamase (ESBL) production was screened using ceftazidime and ceftriaxone disks and confirmed by double-disc synergy and combined disc methods. Among the 737 processed specimens, 76 (10.3%) showed bacterial growth, of these isolates 63 (82.9%) were Gram-negative bacteria. *Escherichia coli* (39.7%) was the most prevalent isolate followed by *Salmonella* Typhi (19.0%). Most Gram-negative isolates showed high resistance to amoxicillin (55.6%) and cefixime (41.3%). Chloramphenicol (96.8%), piperacillin/ tazobactam (95.2%) and meropenem (92.1%) were the most effective antibiotics. Among the 25 *E. coli* isolates, 13 (52%) were multidrug resistant and 12 (48%) were suspected ESBL producers, of which 10 were phenotypically confirmed. The high prevalence of multidrug resistance and ESBL production highlights the need for routine antimicrobial susceptibility testing and continuous surveillance to guide appropriate antibiotic therapy.

Keywords : Gram-negative bacteria, multi drug resistant, ESBL, E. *coli*

Introduction

Antimicrobial resistance has become a significant global public health threat, undermining the effective prevention and treatment of a broad range of infectious

diseases (Weiner et al., 2016). Gram-negative bacteria are of particular concern due to their intrinsic resistance mechanisms, ability to acquire additional resistance genes and their increasing involvement in multidrug-resistant (MDR) infections. These bacteria have a characteristic complex, multilayered cell envelope made of lipopolysaccharide, phospholipids and proteins that act as an effective barrier against many antimicrobial agents and host immune system (Dorman et al., 2016). These pathogens are responsible for a substantial proportion of hospital and community-acquired infections, including urinary tract infections, bloodstream infections, respiratory tract infections, and wound infections (Rasko & Sperandio, 2010; Parajuli et al., 2017).

The management of infections caused by Gram-negative bacteria has become increasingly difficult due to the rapid emergence and spread of resistance to commonly used antimicrobial agents. Easy access to antibiotics often results in misuse, including overuse, underdosing, and treatment of non-bacterial infections, facilitating the emergence of drug-resistant bacteria (Baral et al., 2012). Mechanisms including the production of extended-spectrum β-lactamases (ESBLs), carbapenemases, efflux pump and loss of porins substantially restrict available treatment options (Munita & Arias, 2016).

The escalating use of broad spectrum cephalosporin antibiotics has emerged as a significant factor contributing to the high rate 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, making infections caused by such bacteria increasingly difficult to treat due to resistance to multiple antibiotics (Tooke et al., 2019; Paterson & Bonomo, 2005). Low- and middle-income countries, including Nepal, face a disproportionate burden of AMR owing to factors such as inappropriate antibiotic use, limited diagnostic facilities, lack of antimicrobial stewardship programs, and inadequate infection control practices. In Nepalese healthcare settings, empirical antibiotic therapy is frequently initiated before bacterial culture and susceptibility testing results are available, which may contribute to treatment failure and increased resistance when local resistance patterns are unknown (Bora et al., 2014; Baral et al., 2013; Pariyar et al., 2023).

Periodic surveillance of antimicrobial susceptibility patterns is essential to guide empirical therapy, inform antimicrobial stewardship strategies, and monitor emerging resistance trends. There is a need for continuous monitoring and enhanced surveillance in tertiary care hospitals in Nepal to better understand, detect and track emerging bacterial resistance trends.

In this context, the present study was designed to assess the antimicrobial

susceptibility patterns of Gram-negative bacteria isolated from various clinical samples in a tertiary care hospital in Nepal.

Methodology

Study design and study site

A hospital based cross sectional study was conducted at the Department of Microbiology, Sukraraj Tropical and Infectious Disease Hospital, Teku, Kathmandu, Nepal. The study was conducted over a four-month period from April 2023 to July 2023. Patients of all age groups and both sexes attending the outpatient (OPD) and inpatient (IPD) departments during the study period were included.

Sample collection and processing

A total of 737 clinical specimens, comprising blood (n=263), sputum (n=260) and urine (n=214), were collected and processed for bacterial isolation. Blood and sputum samples were inoculated on Blood Agar (BA) and MacConkey Agar (MA) plates. Urine samples were cultured using semiquantitative method on Cystine Lactose Electrolyte Deficient (CLED) agar. All the plates were incubated aerobically at 37°C for 24 hours. Bacterial isolates were identified using standard microbiological methods, including colony morphology, Gram staining and various biochemical tests according to standard laboratory procedures (Collee et al., 1996).

Antibiotic susceptibility testing

The resistance pattern of the isolates was determined using the modified Kirby–Bauer disk diffusion method on Mueller–Hinton agar in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2016). The antibiotics used in the study were amoxicillin (20 µg), cefixime (5 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), imipenem (10 µg), meropenem (10 µg), nitrofurantoin (300 µg), piperacillin/tazobactam (100/10 µg), cotrimoxazole (25 µg), chloramphenicol (30 µg).

Detection of Multi drug resistance and Extended spectrum β Lactamase producers

Isolates that showed resistance to at least one antibiotic in three or more different antimicrobial classes were classified as MDR.

Isolates showing resistance to third-generation cephalosporins, specifically ceftazidime or ceftriaxone, with inhibition zone diameters of ≤22 mm for ceftazidime and ≤25 mm for ceftriaxone**,** were considered potential ESBL producers and were subsequently subjected to confirmatory testing.

Phenotypic confirmation of ESBL producer

Extended-spectrum β-lactamase (ESBL) production was confirmed by the double-disc diffusion method and the combined disc test (CLSI, 2016).

In DDST method, ceftazidime (30 μg) and cefotaxime (30 μg) were placed 30 mm (center to center) from amoxicillin/clavulanic acid (20 μg /10 μg). A clear

extension of the inhibition zone of cefotaxime toward the amoxicillin–clavulanate disc was considered indicative of ESBL production.

In combined disc method, ≥ 5 mm increase in zone of inhibition for cephalosporin disc combined with clavulanic acid compared to cephalosporin alone indicated ESBL production.

Escherichia coli ATCC 25922 was used as control strain during identification and antibiotic susceptibility testing of organisms.

Data analysis

The data were entered and analyzed using Microsoft Excel. The chi-square test was applied to evaluate the association between variables, and a p-value of ≤ 0.05 was considered statistically significant.

Results and Discussion

Growth positivity among clinical samples

Among the 737 different clinical specimens processed, 76 (10.3%) showed bacterial growth. The majority of samples were obtained from patients aged 20-39 years. The culture positivity rate was highest in the 80-99 age group (21.7%). A statistically significant association was observed between the age group and culture positivity (p=0.008) **(Table 1)**. A higher rate of culture positivity among older age groups has also been reported in previous studies (Nepal et al., 2017; Lee et al., 2018). Age-related alterations in immune function, increased exposure to nosocomial pathogens, and the presence of multiple comorbidities make elderly individuals more susceptible to infections (Rowe & Juthani-Mehta, 2014; Montecino-Rodriguez et al., 2013).

The female to male ratio in the studied population was 1:1.4. Bacterial growth was slightly higher among the male patients (11.9%) compared to the female patients (8.1%). Previous studies have also reported a higher prevalence of bacterial infections among male patients than females (Akhtar et al., 2021; Khurana et al., 2018; Parajuli et al., 2017). Most of the samples were collected from outpatients rather than inpatients. However, the culture positivity rate was higher among the inpatients (14.3%) than the outpatients (10.1%), although this difference was not statistically significant **(Table 1)**. A similar result was observed in a study done at International Friendship Hospital in Kathmandu (Pariyar et al., 2023, Kayastha et al., 2020). In contrast, another similar study from Om Hospital and Research Center, Kathmandu has reported higher proportion of bacterial growth among outpatient samples (Nepal et al., 2017). Such variations may be attributed to differences in the number of samples collected from OPD and IPD settings. Among specimen types, urine samples showed the highest growth rate (35, 13.3%), followed by sputum (26, 10%) and blood samples (15, 7%) **(Table 1)**. The higher bacterial incidence observed in urine samples in this study is consistent with findings from previous studies (Nepal et al.,

2017; Shrestha et al., 2022; Dhungana et al., 2019). Urine is one of the most frequently received specimens in clinical laboratories, and its higher representation in the study may reflect the high prevalence of urinary tract infections in healthcare settings.

Table 1: *Demographic characteristics and bacterial growth in studied population*

Aspects	Total		Growth		P value
	N	%	N	%	
Age group					
≤ 19	62	8.4	8	12.9	0.008
20-39	320	43.4	20	6.2	
40-59	220	29.9	25	11.4	
60-79	112	15.2	18	16.1	
80-99	23	3.1	5	21.7	
Gender					
Male	429	58.2	51	11.9	0.124
Female	308	41.8	25	8.1	
Departments					
Outpatients	702	95.3	71	10.1	0.612
Inpatients	35	4.7	5	14.3	
Specimens					
Urine	263	35.7	35	13.3	0.055
Sputum	260	35.3	26	10.0	
Blood	214	29.0	15	7.0	

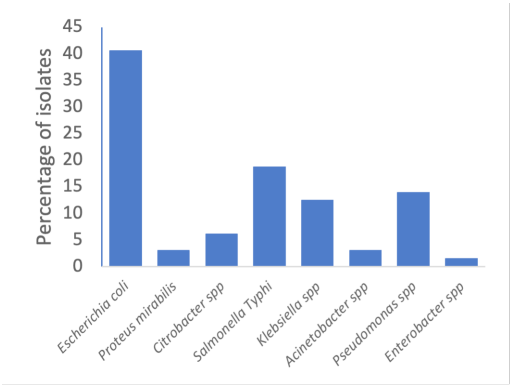

Figure 1: *Distribution of bacterial isolates*

The present study demonstrated that Gram negative bacteria are the predominant pathogens isolated from clinical specimens. Out of 76 bacterial isolates, 63 (82.9%) were Gram-negative bacteria and 13 (17.1%) were Gram-positive bacteria. *E. coli* was the most frequently isolated Gram-negative bacteria (25, 39.7%) followed by *Salmonella* Typhi (12, 19.0%) (**Figure 1**). The predominance of *E. coli* observed in this study is consistent with findings from other studies conducted in Nepal (Pariyar et al., 2023; Tamang et al., 2017; Baral et al., 2012). The higher prevalence of *E. coli* and *Salmonella* Typhi may be attributed to the inclusion of urine and blood samples in the study.

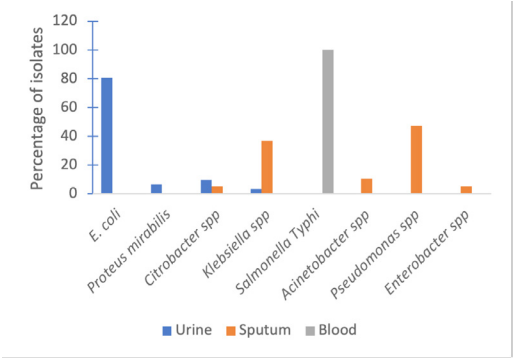

Figure 2: *Bacterial profile of Gram-Negative isolates*

Among 31 urine isolates, *E. coli* was the most frequently identified pathogen (25, 80.6%), followed by *Citrobacter* spp. (3, 9.7%) (**Figure 2**), which aligns with findings from Pariyar et al. (2023), Jalil et al. (2022), and Giri et al. (2020). Being an enteric commensal, *E. coli* can easily ascend from the anus to the urinary tract, leading to bladder infections.

All positive blood cultures yielded *Salmonella* Typhi (15, 100%) (**Figure 2**). Unlike this study, other reports have noted a lower incidence of *S.* Typhi (Ahmad et al., 2023; Mina et al., 2023). The current study was conducted during a period with a high number of typhoid cases, which likely explains the predominance of *S.* Typhi in blood samples.

In sputum specimens, *Pseudomonas* spp (9, 45%) and *Klebsiella* spp (7, 35%) were the dominant isolates (**Figure 2**). Similar trends were reported by Seidler et al. (2016) and Siddalingappa et al. (2017), who observed a high prevalence of *Pseudomonas* spp in respiratory tract infections. *Pseudomonas* spp is a ubiquitous pathogen known for causing a wide range of infections, including hospital-acquired infections (HAIs).

Antibiotic susceptibility profile of Gram-negative isolates

Most of the Gram-negative bacterial isolates were found to be resistant towards amoxicillin (35, 55.6%) and cefixime (26, 41.3%) while chloramphenicol, piperacillin/tazobactam and meropenem were effective to more than 90% isolates **(Table 2)**. Several studies have reported lower resistance rates to meropenem (Bora et al., 2014; Sah et al., 2021; Kayastha et al., 2020). The high resistance of Gram-negative bacteria to amoxicillin observed in this study is consistent with previous findings at Shahid Gangalal Hospital, where 100% resistance was reported (Sah et al., 2021; Pariyar et al., 2023).

Antibiotic susceptibility pattern of *E. coli*

Among the eleven common antibiotics tested against 25 *E. coli* isolates, imipenem, nitrofurantoin and chloramphenicol were found to be effective against (23) 92% isolates. Most of the isolates were resistant to amoxicillin (17, 68.0%) and cotrimoxazole (16, 64.0%) **(Table 3)**. A similar resistance pattern has been documented in earlier studies (Weiner et al., 2016; Niranjan & Malini, 2014; Lee et al., 2018; Giri et al., 2020).

Table 2: *Antibacterial susceptibility pattern of Gram-negative isolates (n=63)*

Antibiotics used	Resistant n (%)	Sensitive n (%)
Amoxicillin (20 µg)	35 (55.6%)	28 (44.4%)
Cefixime (5 µg)	26 (41.3%)	37 (58.7%)
Ceftriaxone (30 µg)	22 (34.9%)	41 (65.1%)
Ciprofloxacin (5 µg)	21 (33.3%)	42 (66.7%)
Gentamicin (10 µg)	8 (12.7%)	55 (87.3%)
Imipenem (10 µg)	8 (12.7%)	55 (87.3%)
Meropenem (10 µg)	5 (7.9%)	58 (92.1%)
Nitrofurantoin (300 µg)	12 (19.0%)	51 (81.0%)
Piperacillin/Tazobactam (100/10 µg)	3 (4.8%)	60 (95.2%)
Cotrimoxazole (25 µg)	24 (38.1%)	39 (61.9%)
Chloramphenicol (30 µg)	2 (3.2%)	61 (96.8%)

Table 3: *Antibiotic susceptibility profile of E. coli (n=25)*

Antibiotics used	Resistant n (%)	Sensitive n (%)
Amoxicillin (20 µg)	17 (68.0%)	8 (32.0%)
Cefixime (5 µg)	15 (60.0%)	10 (40.0%)
Ceftriaxone (30 µg)	14 (56.0%)	11 (44.0%)
Ciprofloxacin (5 µg)	14 (56.0%)	11 (44.0%)
Gentamicin (10 µg)	3 (12.0%)	22 (88.0%)
Imipenem (10 µg)	2 (8.0%)	23 (92.0%)
Meropenem (10 µg)	3 (12.0%)	22 (88.0%)
Nitrofurantoin (300 µg)	2 (8.0%)	23 (92.0%)
Piperacillin/Tazobactam (100/10 µg)	5 (20.0%)	20 (80.0%)
Cotrimoxazole (25 µg)	16 (64.0%)	9 (36.0%)
Chloramphenicol (30 µg)	2 (8.0%)	23 (92.0%)

Multi drug resistance and ESBL production

The study revealed a considerable prevalence of MDR and ESBL producing *E. coli.* Among the 25 *E. coli* isolates, 13 were identified as multidrug-resistant (MDR), with eight isolates exhibiting resistance to four different classes of antibiotics (**Figure 3**). The increasing resistance is attributed to the acquisition of diverse resistance genes, production of various β-lactamase enzymes, activity of multiple efflux pumps, and reduced drug uptake by bacteria. Comparable MDR patterns have been reported in previous studies from Nepal (Pariyar et al., 2023; Parajuli et al., 2017; Shrestha et al., 2022;

Dhungana et al., 2019). MDR represents a global challenge and poses a significant obstacle for clinicians in treating infections caused by such organisms. In low-income countries like Nepal, indiscriminate and excessive use of antibiotics contributes to the emergence and spread of MDR strains in clinical settings.

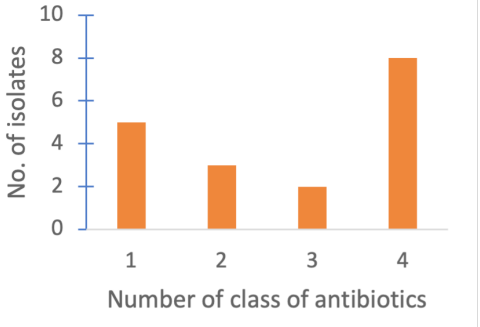

Figure 3: *E. coli resistant to number of antibiotic classes*

Out of the isolates, 12 were identified as ESBL producers, and 10 of these were confirmed by the double-disk synergy test (DDST) and combined disc method. ESBL producing organisms pose a significant challenge to clinical therapy. The high number of ESBL producers observed in this study is consistent with previous reports from Nepal (Raut et al., 2015; Nepal et al., 2017; Pokharel et al., 2006; Shrestha et al., 2022). The prevalence of ESBL-producing *E. coli* varies widely across countries and institutions. In Asia, reported rates are 4.8% in Korea, 8.5% in Taiwan, and up to 12% in Hong Kong (Ho et al., 2000; Pai et al., 1999; Yan et al., 2000). In India, the prevalence ranges from 22% to 75% (Kumar & Malini, 2013), while in Japan it is <0.1% (Yagi et al., 2000). In the United States, ESBL-producing *E. coli* accounts for 0–25% of isolates, with an average of approximately 3% (NNIS, 2004).

A key limitation of this study was that only disc diffusion was performed for resistance analysis, while Minimun Inhibitory Concentration (MIC) determination could have provided more accurate results. Other potential mechanisms of resistance were not investigated.

Conclusion

The predominant pathogens isolated from the clinical specimens in this study were Gram-negative bacteria, with *Escherichia coli* being the most frequently recovered organism. A considerable proportion of isolates exhibited multi drug resistance and ESBL production posing a substantial challenge for effective antimicrobial therapy. Continuous surveillance of antimicrobial susceptibility patterns and routine screening for ESBL production are essential to guide appropriate antibiotic therapy and strengthen antimicrobial stewardship programs in healthcare settings.

Authors contribution

AA: writing original draft, investigation, data management, formal analysis; SUP: writing original draft, investigation; NP: writing original draft, investigation; RG: writing original draft, investigation; SM: supervision, conceptualization, data management, writing-review and editing.

Declaration of competing interest

The authors declare that they have no known competing interest.

References

Ahmad, M., Shah, N., & Siddiqui, M. A. (2023). Frequency and Antibiotics Sensitivity Pattern of Culture-Positive *Salmonella* Typhi in Children. *Journal of the College of Physicians and Surgeons-Pakistan : JCPSP*, *33*(3), 303–307. https://doi.org/10.29271/jcpsp.2023.03.303

Akhtar, A., Hassali, M. A. A., Zainal, H., Ali, I., Iqbal, M. S., & Khan, A. H. (2021). Respiratory-tract infections among geriatrics: prevalence and factors associated with the treatment outcomes. *Therapeutic advances in respiratory disease*, *15*, 1753466620971141. https://doi.org/10.1177/1753466620971141

Baral, P., Neupane, S., Marasini, B. P., Ghimire, K. R., Lekhak, B., & Shrestha, B. (2012). High prevalence of multidrug resistance in bacterial uropathogens from Kathmandu, Nepal. *BMC research notes*, *5*, 38. https://doi.org/10.1186/1756-0500-5-38

Baral, P., Neupane, S., Shrestha, B., Ghimire, K. R., Marasini, B. P., & Lekhak, B. (2013). Clinical and microbiological observational study on AmpC β-lactamase-producing Enterobacteriaceae in a hospital of Nepal. *The Brazilian journal of infectious diseases : an official publication of the Brazilian Society of Infectious Diseases*, *17*(2), 256–259. https://doi.org/10.1016/j.bjid.2012.09.012

Bora, A., Sanjana, R., Jha, B. K., Mahaseth, S. N., & Pokharel, K. (2014). Incidence of metallo-beta-lactamase producing clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* in central Nepal. *BMC research notes*, *7*, 557. https://doi.org/10.1186/1756-0500-7-557

CLSI (2016). Performance Standards for Antimicrobial Susceptibility Testing; Twenty fourth Informational Supplement (M100-S28). Wayne PA: CLSI; 2016.

Collee, J. G., Miles, R. S., & Watt, B. (1996) Tests for Identification of Bacteria. In: Collee, J. G., Fraser, A. G., Marmion, B. P., & Simmons, A., editors. Mackie and McCartney Practical Medical Microbiology. 14. New York: Churchill Livingstone, 131–149.

Dhungana, K., Awal, B. K., Dhungel, B., Sharma, S., Banjara, M. R., & Rijal, K. R. (2019). Detection of *Klebsiella pneumoniae* Carbapenemase (KPC) and Metallo-Beta Lactamase (MBL) producing Gram negative bacteria isolated from different clinical samples in a transplant center, Kathmandu, Nepal. *Acta Sci Microbiol. 2*, 60-69. DOI: 10.31080/ASMI.2019.02.0432

Dorman, C. J., Colgan, A., & Dorman, M. J. (2016). Bacterial pathogen gene regulation: a DNA-structure-centred view of a protein-dominated domain. *Clinical science (London, England : 1979)*, *130*(14), 1165–1177. https://doi.org/10.1042/CS20160024

Giri, A., Kafle, R., Singh, G. K., & Niraula, N. (2020). Prevalence of E. Coli in Urinary Tract Infection of Children Aged 1-15 Years in A Medical College of Eastern Nepal. *JNMA; journal of the Nepal Medical Association*, *58*(221), 11–14. https://doi.org/10.31729/jnma.4796

Ho, P. L., Tsang, D. N., Que, T. L., Ho, M., & Yuen, K. Y. (2000). Comparison of screening methods for detection of extended-spectrum beta-lactamases and their prevalence among

Escherichia coli and *Klebsiella* species in Hong Kong. *APMIS : acta pathologica, microbiologica, et immunologica Scandinavica*, *108*(3), 237–240. https://doi.org/10.1034/j.1600-0463.2000.d01-50.x

Jalil, M. B., & Al Atbee, M. Y. N. (2022). The prevalence of multiple drug resistance *Escherichia coli* and *Klebsiella pneumoniae* isolated from patients with urinary tract infections. *Journal of clinical laboratory analysis*, *36*(9), e24619. https://doi.org/10.1002/jcla.24619

Kayastha, K., Dhungel, B., Karki, S., Adhikari, B., Banjara, M. R., Rijal, K. R., & Ghimire, P. (2020). Extended-Spectrum β-Lactamase-Producing *Escherichia coli* and *Klebsiella* Species in Pediatric Patients Visiting International Friendship Children's Hospital, Kathmandu, Nepal. *Infectious diseases*, *13*, 1178633720909798. https://doi.org/10.1177/1178633720909798

Khurana, S., Bhardwaj, N., Kumari, M., Malhotra, R., & Mathur, P. (2018). Prevalence, etiology, and antibiotic resistance profiles of bacterial bloodstream infections in a tertiary care hospital in Northern India: A 4-year study. *Journal of laboratory physicians*, *10*(4), 426–431. https://doi.org/10.4103/JLP.JLP_78_18

Kumar, C. N., & Mahadeva, M. S. (2013). Extended spectrum beta lactamases in uropathogen. *Asian Journal of Pharmaceutical and Clinical Research*, *6* (3), 207-210.

Lee, D. S., Lee, S. J., & Choe, H. S. (2018). Community-Acquired Urinary Tract Infection by *Escherichia coli* in the Era of Antibiotic Resistance. *BioMed research international*, *2018*, 7656752. https://doi.org/10.1155/2018/7656752

Mina, S. A., Hasan, M. Z., Hossain, A. K. M. Z., Barua, A., Mirjada, M. R., & Chowdhury, A. M. M. A. (2023). The Prevalence of Multi-Drug Resistant *Salmonella* typhi Isolated From Blood Sample. *Microbiology insights*, *16*, 11786361221150760. https://doi.org/10.1177/11786361221150760

Montecino-Rodriguez, E., Berent-Maoz, B., & Dorshkind, K. (2013). Causes, consequences, and reversal of immune system aging. *The Journal of clinical investigation*, *123*(3), 958–965. https://doi.org/10.1172/JCI64096

Munita, J. M., & Arias, C. A. (2016). Mechanisms of Antibiotic Resistance. *Microbiology spectrum*, *4*(2), 10. 1128/microbiolspec. VMBF-0016-2015. https://doi.org/10.1128/microbiolspec.VMBF-0016-2015

NNIS (2004). National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004. *American journal of infection control*, *32*(8), 470–485. https://doi.org/10.1016/S0196655304005425

Nepal, K., Pant, N. D., Neupane, B., Belbase, A., Baidhya, R., Shrestha, R. K., Lekhak, B., Bhatta, D. R., & Jha, B. (2017). Extended spectrum beta-lactamase and metallo beta-lactamase production among *Escherichia coli* and *Klebsiella pneumoniae* isolated from different clinical samples in a tertiary care hospital in Kathmandu, Nepal. *Annals of clinical microbiology and antimicrobials*, *16*(1), 62. https://doi.org/10.1186/s12941-017-0236-7

Niranjan, V., & Malini, A. (2014). Antimicrobial resistance pattern in *Escherichia coli* causing urinary tract infection among

inpatients. *The Indian journal of medical research*, *139*(6), 945–948.

Pai, H., Lyu, S., Lee, J. H., Kim, J., Kwon, Y., Kim, J. W., & Choe, K. W. (1999). Survey of extended-spectrum beta-lactamases in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*: prevalence of TEM-52 in Korea. *Journal of clinical microbiology*, *37*(6), 1758–1763. https://doi.org/10.1128/JCM.37.6.1758-1763.1999.

Parajuli, N. P., Acharya, S. P., Mishra, S. K., Parajuli, K., Rijal, B. P., & Pokhrel, B. M. (2017). High burden of antimicrobial resistance among gram negative bacteria causing healthcare associated infections in a critical care unit of Nepal. *Antimicrobial resistance and infection control*, *6*, 67. https://doi.org/10.1186/s13756-017-0222-z.

Pariyar, M., Adhikari, S., Regmi, R. S., Dhungel, B., Banjara, M. R., Rijal, B. P., Rijal, K. R., & Ghimire, P. (2023). Beta-Lactamase-Producing Gram-Negative Bacterial Isolates Among the Patients Attending a Tertiary Care Hospital, Kathmandu, Nepal. *Microbiology insights*, *16*, 11786361221150761. https://doi.org/10.1177/11786361221150761.

Paterson, D. L., & Bonomo, R. A. (2005). Extended-spectrum beta-lactamases: a clinical update. *Clinical microbiology reviews*, *18*(4), 657–686. https://doi.org/10.1128/CMR.18.4.657-686.2005

Pokharel, B. M., Koirala, J., Mishra, S. K., Dahal, R. K., Khadga, P. K. & Tuladhar, N. R. (2006). Multidrug resistance and extended spectrum beta-lactamase producing strains causing lower respiratory tract and urinary tract infection. *Journal of Institute of Medicine, 28*(3)*,* 19-27.

Rasko, D. A., & Sperandio, V. (2010). Anti-virulence strategies to combat bacteria-mediated disease. *Nature reviews. Drug discovery*, *9*(2), 117–128. https://doi.org/10.1038/nrd3013

Raut, S., Gokhale, . S. & Adhikari, . B. (2015) Prevalence of Extended Spectrum Beta-Lactamases among *Escherichia coli* and *Klebsiella* spp isolates in Manipal Teaching Hospital, Pokhara, Nepal. *Journal of Microbiology and Infectious Diseases*, 5 (2), 69-75. https://doi.org/10.5799/shinjs.02.2015.02.0179

Rowe, T. A., & Juthani-Mehta, M. (2014). Diagnosis and management of urinary tract infection in older adults. *Infectious disease clinics of North America*, *28*(1), 75–89. https://doi.org/10.1016/j.idc.2013.10.004

Sah, R. S. P., Dhungel, B., Yadav, B. K., Adhikari, N., Thapa Shrestha, U., Lekhak, B., Banjara, M. R., Adhikari, B., Ghimire, P., & Rijal, K. R. (2021). Detection of *TEM* and *CTX-M* Genes in *Escherichia coli* Isolated from Clinical Specimens at Tertiary Care Heart Hospital, Kathmandu, Nepal. *Diseases (Basel, Switzerland)*, *9*(1), 15. https://doi.org/10.3390/diseases9010015

Seidler, D., Griffin, M., Nymon, A., Koeppen, K., & Ashare, A. (2016). Throat Swabs and Sputum Culture as Predictors of *P. aeruginosa* or *S. aureus* Lung Colonization in Adult Cystic Fibrosis Patients. *PloS one*, *11*(10), e0164232. https://doi.org/10.1371/journal.pone.0164232

Shrestha, A., Acharya, J., Amatya, J., Paudyal, R., & Rijal, N. (2022). Detection of Beta-Lactamases (ESBL and MBL) Producing Gram-Negative Pathogens in National Public Health Laboratory

of Nepal. *International journal of microbiology*, *2022*, 5474388. https://doi.org/10.1155/2022/5474388

Siddalingappa, C. M., Kalpana, L., Sagar, P., Vasudha, T. K., & Acharya, A. (2017). Sensitivity Pattern of Bacteria Causing Respiratory Tract Infections in a Tertiary Care Centre. *International Journal of Basic & Clinical Pharmacology, 2*(5), 590-595. https://www.ijbcp.com/index.php/ijbcp/article/view/1325.

Tamang, K., Shrestha, P., Koirala, A., Khadka, J., Gautam, N., & Rijal, K. R. (2017). Prevalence of bacterial uropathogens among diabetic patients attending Padma Nursing Hospital of Western Nepal. *Himal J Sci Technol, 1*(1), 15-19.

Tooke, C. L., Hinchliffe, P., Bragginton, E. C., Colenso, C. K., Hirvonen, V. H. A., Takebayashi, Y., & Spencer, J. (2019). β-Lactamases and β-Lactamase Inhibitors in the 21st Century. *Journal of molecular biology*, *431*(18), 3472–3500. https://doi.org/10.1016/j.jmb.2019.04.002

Weiner, L. M., Webb, A. K., Limbago, B., Dudeck, M. A., Patel, J., Kallen, A. J., Edwards, J. R., & Sievert, D. M. (2016). Antimicrobial-Resistant Pathogens Associated With Healthcare-Associated Infections: Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011-2014. *Infection control and hospital epidemiology*, *37*(11), 1288–1301. https://doi.org/10.1017/ice.2016.174

Yagi, T., Kurokawa, H., Shibata, N., Shibayama, K., & Arakawa, Y. (2000). A preliminary survey of extended-spectrum beta-lactamases (ESBLs) in clinical isolates of *Klebsiella pneumoniae* and Escherichia *coli* in Japan. *FEMS microbiology letters*, *184*(1), 53–56. https://doi.org/10.1111/j.1574-6968.2000.tb08989.x

Yan, J. J., Wu, S. M., Tsai, S. H., Wu, J. J., & Su, I. J. (2000). Prevalence of SHV-12 among clinical isolates of *Klebsiella pneumoniae* producing extended-spectrum beta-lactamases and identification of a novel AmpC enzyme (CMY-8) in Southern Taiwan. *Antimicrobial agents and chemotherapy*, *44*(6), 1438–1442. https://doi.org/10.1128/AAC.44.6.1438-1442.2000