

Occurrence of ESKAPEE pathogens in blood samples of patients with bloodstream infections and their antimicrobial resistance patterns

Manandhar R,¹ Neupane S,¹ Lama R,² Mahto M,³ Raghubanshi BR¹

¹Ruchee Manandhar,¹Sweekrity Neupane,¹Bijendra Raj Raghubanshi, Department of Microbiology, KIST Medical College and Teaching Hospital, Imadol Lalitpur; ²Rajni Lama, Department of Microbiology, Patan Academy of Health Sciences, Patan, Lalitpur; ³Moni Mahto, Department of Microbiology, Peoples Dental College and Teaching Hospital, Nayabazar, Kathmandu, Nepal.

ABSTRACT

Introduction: Some of the most threatening drug-resistant microbes are the members of ESKAPEE group, an acronym for *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp., and *Escherichia coli* which are posing a constant risk with increasing treatment challenges worldwide.

Objectives: To determine the occurrence of ESKAPEE pathogens in blood samples of patients attending and determine their antimicrobial resistance pattern.

Methodology: This hospital based cross-sectional study was conducted in the Department of Microbiology, KIST Medical College Teaching Hospital retrospectively. The records were obtained from culture and sensitivity test performed on all the blood samples received in the Microbiology Laboratory from July 2023- June 2024. The data was entered and analyzed in WHONET 2024 program.

Results: Out of 4180 blood samples received, pathogenic bacteria were isolated from 108 samples. ESKAPEE pathogens constituted 68.5% of total bacterial isolates. Five (6.75%) *Enterococcus* spp, 33 (44.59%) *Staphylococcus aureus*, 10 (13.5%) *Klebsiella pneumoniae*, 9 (12.16%) *Acinetobacter* spp., 3 (4.05%) *Pseudomonas aeruginosa*, 1 (1.35%) *Enterobacter* spp and 13 (17.56%) *Escherichia coli* were isolated. Sixty three percent *Staphylococcus aureus* were Methicillin resistant. Twenty percent *Klebsiella pneumoniae* and 23% *Escherichia coli* were Extended-Spectrum Beta-Lactamases producers. Sixty percent of *Klebsiella pneumoniae* and 22.22% *Acinetobacter* spp. showed carbapenem resistance.

Conclusion: Our study shows increased prevalence of ESKAPEE pathogens resistant to commonly used antibiotics. Judicious use of antimicrobials, active infection control practices, stringent antibiotic policy and regular surveillance is the need of the hour for preventing antimicrobial resistance.

Keywords: Antimicrobial resistance; Bloodstream infections; ESKAPEE; Multidrug resistance

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Address for correspondence

Dr. Ruchee Manandhar
Associate Professor, Department of Microbiology
KIST Medical College and Teaching Hospital,
Imadol, Lalitpur, Nepal.
E-mail: rucheeamanandhar@gmail.com

INTRODUCTION

The ESKAPE pathogens are a group of infectious bacteria first defined by Rice in 2008 and consist of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.¹ This group is often extended to "ESKAPEE" to include *Escherichia coli* due to its increasing prevalence, ability to acquire resistance genes and escape the effects of standard antibiotics.^{2,3} The emergence of these pathogens with highly resistant

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phenotypes have highlighted the urgency of the problem as infections with multidrug resistant bacteria are associated with higher mortality, longer hospital stays, and increased healthcare costs.⁴

Despite recent innovations, bloodstream infections remain challenging to treat.⁵ Because of the high burden of untreated infection, clinicians administer antimicrobial drugs in patients suspected of bloodstream infections at rates of 50%–70%, far exceeding the actual infection rate of 10%–15%.⁶ A consequence of overprescribing antibiotics is the emergence and spread of antibiotic resistance.

Continuous surveillance is an essential tool for understanding the magnitude and future trends of antimicrobial resistance needed for patient management and preparing future strategies for antibiotic usage and infection control procedures.^{6,7} Our study therefore intends to present the local epidemiology and guide the clinicians to follow evidence based antibiotic policies.

METHODOLOGY

This hospital based cross-sectional study was conducted in the Department of Microbiology, KIST Medical College Teaching Hospital (KISTMCTH) Nepal retrospectively. The records were obtained from culture and sensitivity test performed on all the blood samples received in the Clinical Microbiology Laboratory from July 2023–June 2024 after obtaining ethical clearance from the Institutional Review Committee of KISTMCTH. (IRC REF NO: 2081/82/05)

A total of 4180 blood samples received in the Microbiology lab during the study period (*i.e.*, July 2023 to June 2024) from patients attending different departments of KISTMCTH, including the wards and Out Patient Departments with the clinical suspicion (signs and symptoms) of bloodstream infections were included in the study. Unlabeled and mislabeled samples, samples received after 24 hours of collection, repeated samples were excluded from the study. Sample Size was calculated using the formula:

$$\begin{aligned} \text{Sample size (n)} &= Z^2 \times p \times q / e^2 \\ &= (1.96)^2 \times 0.064 \times (1-0.064) / (0.05)^2 \\ &= 92 \end{aligned}$$

Where,

n= required sample size

Z=1.96 at 95% confidence interval

p= prevalence of ESKAPEE pathogens in blood samples *i.e.* 6.4%⁸

q= 1-p

e= margin of error, 5%

The minimum sample size calculated was 92. Nevertheless, 108 samples with bacterial growth were further studied.

The data recorded in the routine blood culture register in the microbiology laboratory was collected for this. For culture and sensitivity tests, blood sample (5–10ml) collected after proper disinfection of the site were inoculated into automated blood culture bottles. The bottles were incubated in the BC60. Autobio Diagnostic Co. Ltd automated blood culture systems. The bottles were incubated for 7 days. Any bottles where growth was indicated were subcultured in Blood Agar, Chocolate agar and Mac Conkey agar. The subculture plates were checked for growth of bacteria after 24 hours of incubation. Bacterial identification was done based on routine microbiological procedures. Kirby-Bauer disc diffusion method was done for antimicrobial susceptibility tests. The data was entered and analyzed in the WHONET 2024 program.

RESULTS

A total of 4180 blood samples were obtained during the study period among them 108 (2.5%) had growth of pathogenic bacteria. Among the total bacterial growth 74 (68.5%) were ESKAPEE pathogens. Non ESKAPEE pathogens constituted 31.4% of total bacterial isolates, which included *Salmonella Typhi*, *Salmonella Paratyphi A*, Coagulase-negative *Staphylococcus spp.* and *Citrobacter spp.* One *Candida non albicans* species was also isolated (Fig. 1).

The most frequent ESKAPEE pathogen isolated was 33 (44.59%) followed by *Escherichia coli* 13 (17.56%), *Klebsiella pneumoniae* 10 (13.5%), *Acinetobacter spp.* 9 (12.16%), *Staphylococcus aureus* 5 (6.75%), *Pseudomonas aeruginosa* 3 (4.05%) and *Enterobacter spp.* 1 (1.35%). Among the isolated *Staphylococcus aureus* 21 (63.63%) were Methicillin-resistant *Staphylococcus aureus* and 12 (36.36%) were Methicillin-sensitive *Staphylococcus aureus* (Fig 2)

Out of 74 blood samples with ESKAPEE pathogen growth, 49 (66.2%) samples belonged to males and 25 (33.8%) samples belonged to female (Fig 3).

Greater proportion 27 (36.5%) of growths of ESKAPEE pathogens were observed in samples of younger age group *i.e.*, 0-20 years (Table 1).

Antibiotic susceptibility test showed that Enterococcus spp. was sensitive to most antibiotics. Twenty percent of Enterococcus spp. were resistant to Ampicillin. Two out of 5 Enterococcus spp. were resistant to Erythromycin. No Vancomycin-resistant Enterococcus spp. was isolated. Among the *Staphylococcus aureus* isolated, 21(63.6%) were Methicillin resistant. Sixty one percent of MRSA were resistant to Erythromycin. Methicillin- resistant *Staphylococcus aureus* were found to be most sensitive to aminoglycosides (Table 2). Sixty six percent of Methicillin-sensitive *Staphylococcus aureus* were resistant to Ampicillin. Chloramphenicol and aminoglycosides, which included Gentamicin and Amikacin, were found to be most effective drugs for Gram positive ESKAPEE isolates (Table 2 and Fig 4).

Among the Gram negative ESKAPEE isolates, *Klebsiella pneumoniae* were most resistant to Cefotaxime. Among 9 Acinetobacter spp. 5(55.5%), showed resistance to Ceftazidime C 3 (33.3%) to hloramphenicol and 2(22.2%) to Cefotaxime. Sixty six percent of *Pseudomonas aeruginosa* showed resistance to

Meropenem, Ciprofloxacin, Amikacin and Ceftazidime. The only Enterobacter spp. isolated was resistant to Gentamicin, Amikacin, Ciprofloxacin and Cotrimoxazole. The Enterobacter spp. was sensitive to Ceftazidime, Chloramphenicol and Ofloxacin. Out of 13 *Escherichia coli* isolates All were resistant to Ampicillin and 12 (92%) were resistant to Cefotaxime whereas no Carbapenem resistance was observed (Table 4).

Sixty percent of *Klebsiella pneumoniae* and 2 (22.22%) of Acinetobacter spp. showed carbapenem resistance. Two ESBL producing *Klebsiella pneumoniae* were also isolated. Out of which one *Klebsiella pneumoniae* showed pan drug resistance. Twenty three percent of *Escherichia coli* were ESBL producers. All *Escherichia coli* isolates were sensitive to Meropenem. Chloramphenicol followed by Amikacin was found to be most effective drug for Gram negative isolates. (Table 3 and Fig 5).

Table 1: Age wise distribution of the ESKAPEE pathogens

Age group	ESKAPEE pathogens isolated n (%)
0- 20years	27 (36.5)
21-40 years	11 (14.8)
41-60 years	19 (25.6)
61-80 years	14 (19)
>80 years	3 (4)
TOTAL	74

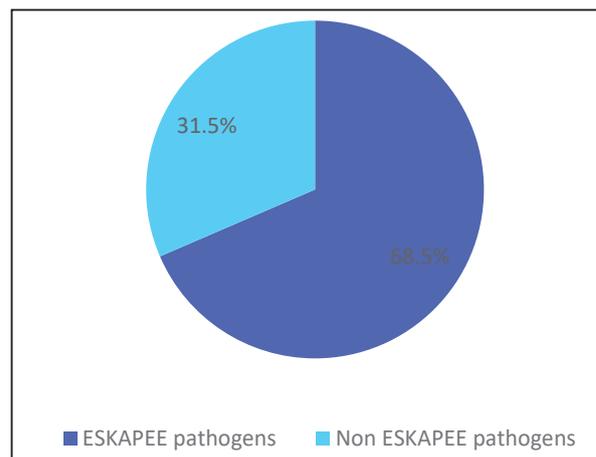


Figure 1: Distribution of Bacterial isolates

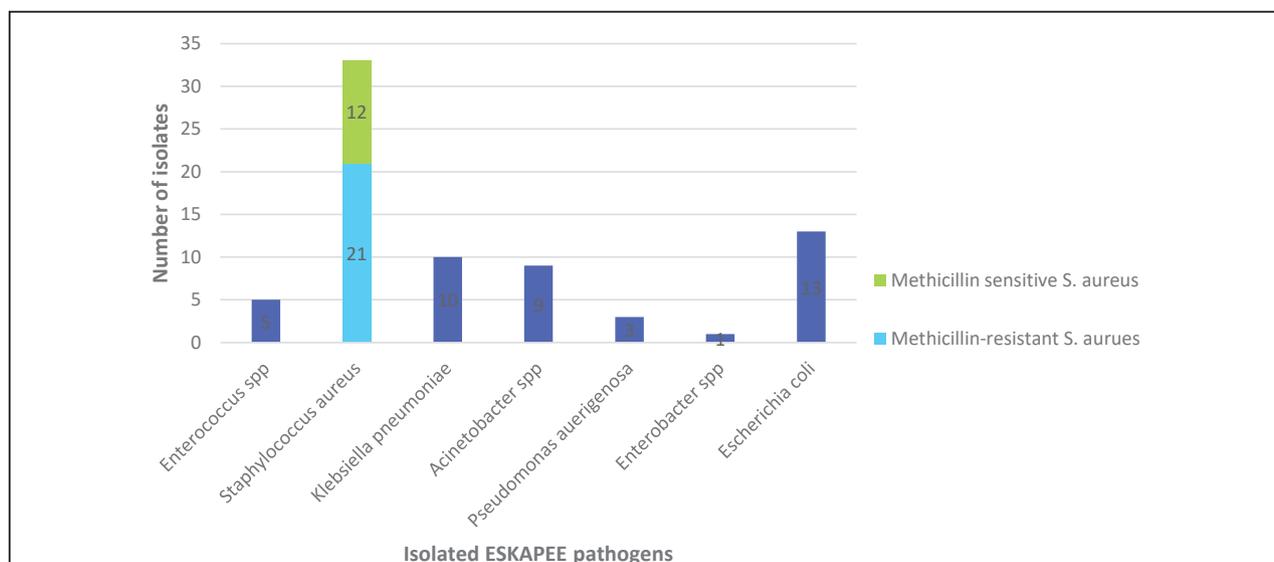


Figure 2: Distribution of ESKAPEE pathogens isolated

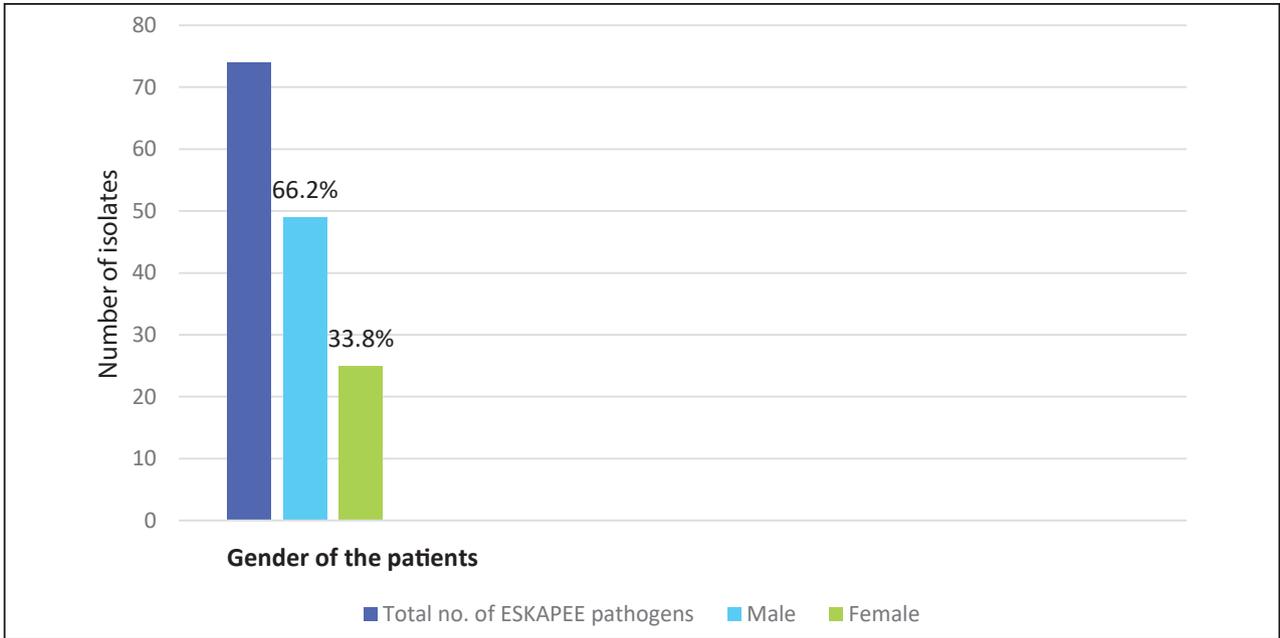


Figure 3: Gender wise distribution of ESKAPEE pathogens

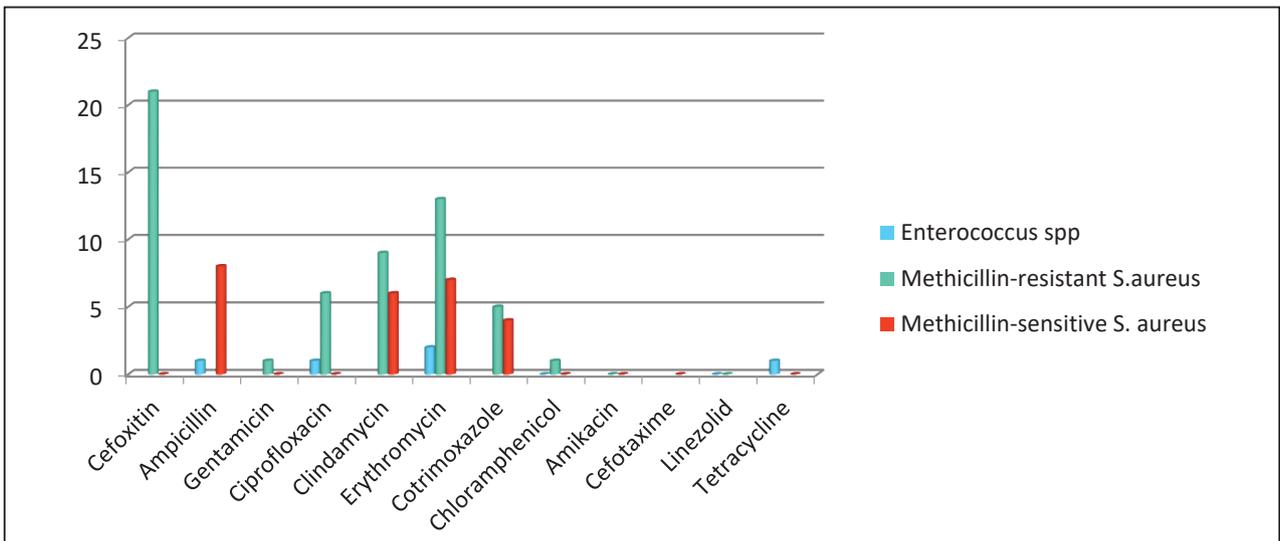


Figure 4: Antibiotic resistance pattern of Gram positive ESKAPEE pathogens

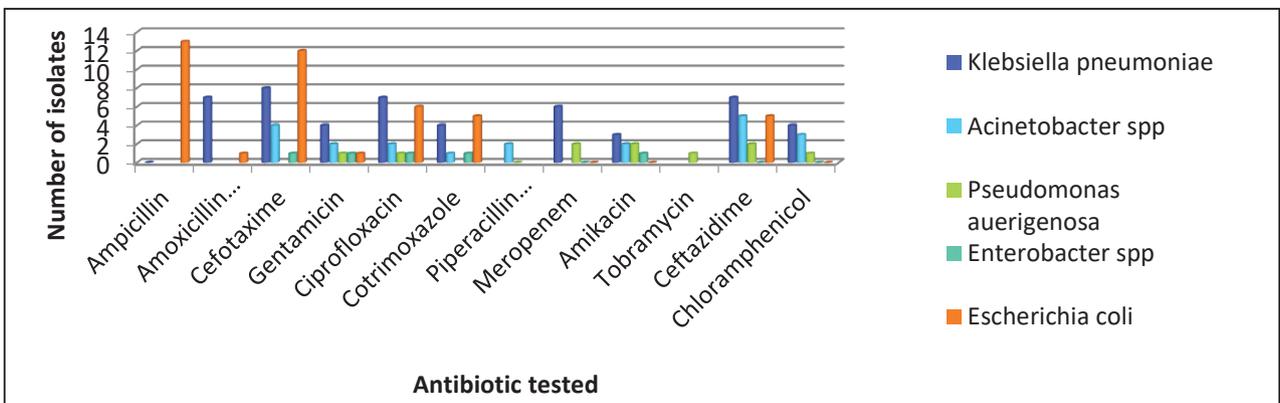


Figure 5: Antibiotic resistance pattern of Gram negative ESKAPEE pathogens

Table 2: Antibigram of Gram positive ESKAPEE pathogens

Name of the isolates	No. of isolates	Antibiotic resistance pattern n (%)										
		Ampicillin	Ampicillin sulbactam	Gentamicin	Ciprofloxacin	Clindamycin	Erythromycin	Cotrimoxazole	Chloramphenicol	Amikacin	Cefotaxime	Linezolid
Enterococcus spp	5	1 (20)	I.R	1 (20)	I.R	2 (40)	I.R	0 (0)	I.R	I.R	0 (0)	1 (20)
Methicillin-resistant Staphylococcus aureus (MRSA)	21	I.R	1 (4.7)	6 (28.5)	9 (42.8)	13 (61.9)	5 (23.8)	1 (4.7)	0 (0)	I.R	0 (0)	I.R
Methicillin-sensitive Staphylococcus aureus (MSSA)	12	0 (0)	8 (66.6)	0 (0)	6 (50)	7 (58.3)	4 (33.3)	0 (0)	0 (0)	0 (0)	-	0 (0)
Staphylococcus aureus	33	21 (63.6)	29 (87)	6 (18.8)	15 (45.4)	20 (60.6)	9 (27.7)	1 (3.03)	0 (0)	21 (63.6)	0 (0)	21 (63.6)

I. R= intrinsic resistance

Table 3: Antibigram of Gram negative ESKAPEE pathogens

S. No. the isolates	Name of the isolates	No. of isolates	Antibiotic resistance pattern n (%)											
			Ampicillin	Amoxicillin clavulanic acid	Cefotaxime	Gentamicin	Ciprofloxacin	Cotrimoxazole	Piperacillin tazobactam	Meropenem	Amikacin	Tobramycin	Ceftazidime	Chloramphenicol
1.	Klebsiella pneumoniae	10	I.R	7 (70)	8 (80)	4 (40)	7 (70)	4 (40)	-	6 (60)	3 (30)	-	7 (70)	4 (40)
2.	Acinetobacter spp	9	I.R	I.R	4 (44.4)	2 (22.2)	2 (22.2)	1 (11.11)	-	2 (22.2)	2 (22.2)	-	5 (55.5)	3 (33.3)
3.	Pseudomonas aeruginosa	3	I.R	I.R	I.R	1 (33.3)	1 (33.3)	I.R	0 (0)	2 (66.6)	2 (66.6)	1 (33.3)	2 (66.6)	1 (33.3)
4	Enterobacter spp	1	I.R	I.R	1 (100)	1 (100)	1 (100)	1 (100)	-	0 (0)	1 (100)	-	0 (0)	0 (0)
5.	Escherichia coli	13	13 (100)	1 (7.6)	12 (92.3)	1 (7.6)	6 (46.1)	5 (38.4)	-	0 (0)	0 (0)	-	5 (38.4)	0 (0)

I. R= intrinsic resistance

DISCUSSION

Infections caused by multidrug-resistant bacteria pose a great challenge to patient care. There has been a drastic increase in the number of MDR bacteria particularly in ESKAPEE group in recent years which has increased morbidity, mortality with significant effect on length of hospital stay and increased financial burden to the patient.^{1,5}

Our study shows the prevalence rate of ESKAPEE pathogens in bloodstream infections to be 68.5% of total bacterial isolates which was similar to a study conducted in Brazil.⁹ A five year analytic study conducted in Turkey showed a lesser (49.4%) prevalence of ESKAPEE pathogens in bloodstream infections which was found to be in increasing trend over the years.⁵ In a study conducted in Tribhuvan University Teaching Hospital, the prevalence of ESKAPEE pathogens isolated from different samples was found to be 32.1%.¹⁰

Staphylococcus aureus was the most frequent ESKAPEE pathogen isolated in our study (44.5%) which was in contrast to studies conducted by Kritsotakis *et al*¹¹ and Ceballos Vela *et al*¹² who reported *Escherichia coli* and *Klebsiella pneumoniae* (22.5%) to be the most common ESKAPEE isolate respectively. Like in our study, Enterobacter spp. were the least frequently isolated bacteria in blood stream infections in studies conducted in Brazil⁹ and Thailand.⁷ This disparity could be because of the variations in geographic conditions and population distribution.

In a retrospective study conducted in China, ESKAPEE were attributable to a significant number (65.3%) of bloodstream infections in patients above 14 years of age¹³ which was in contrast to our study where majority of ESKAPEE pathogens were isolated from patients below 20 years of age. The high prevalence of bloodstream

infections in this age group may be because it includes infants and children having poor skin integrity with developing immune system who frequently participate in activities that predispose them to external environment making them prone to bacterial infections.¹⁴

Tishyadhigama *et al.* reported that the prevalence of MRSA remained stable at 26% from 2000 to 2005.¹⁵ However, a higher estimate of MRSA (65%) has been shown among intensive care unit patients¹⁶ which was similar to our study which reported 65% Methicillin-resistant *Staphylococcus aureus*. No Vancomycin resistant enterococci was isolated in our study similar to a study conducted by Ruekit *et al.*⁷ In our study, 60% *Klebsiella pneumoniae* and 22.22% Acinetobacter spp. showed carbapenem resistance which was similar to a study conducted by Yardimci *et al.*⁵ Enterobacter spp. showed maximum multidrug resistance similar to a study conducted in Thailand.⁷ Twenty percent of *Klebsiella pneumoniae* were ESBL producers which was similar to a study conducted in Shahid Gangalal National Heart Centre, Kathmandu, which reported its prevalence as 25%.¹⁷

CONCLUSION

Our study shows increased prevalence of ESKAPEE pathogens with marked resistance to commonly used antibiotics. Therefore, judicious use of antimicrobials, strict antibiotic policy, active infection control practices, and continuous surveillance should be followed to control the spread of these superbugs.

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