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Original Research Article

Deciphering the distribution of ESKAPE pathogens in various clinical samples and its pattern of antimicrobial resistance: a study from a tertiary care center in Wayanad, Kerala

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ABSTRACT

Background: ESKAPE includes 6 nosocomial pathogens which are capable of 'escaping' the biocidal actions of antimicrobial agents. Antimicrobial resistance of ESKAPE pathogens in clinical samples is crucial for effective patient management and development of targeted treatment strategy. So, the present study was designed to investigate the distribution of ESKAPE pathogens across diverse clinical samples and to find out their prevalence in different clinical sample such as pus, sputum and urine. This provides an insight to their varied ecological niches within healthcare settings.
Methods: This was a retrospective study done at Dr. Moopen's Medical College. A total of 6492 clinical samples were studied to isolate ESKAPE pathogens. Which included urine, sputum and pus samples.
Results: A total of n=6,492 Individual bacteria isolates were recovered from clinical specimens such as sputum (1473), urine (3710) and pus (1309) out of which n=1181 were ESKAPE pathogen. The most frequent clinical sample were pus 580 (49.12%) followed by urine 265 (22.43%) and respiratory samples 336 (28.45%). The most predominant bacteria were found to be *Staphylococcus aureus* (31.39%) in pus whereas *Klebsiella pneumoniae* in urine (40.10%) and respiratory samples (40.48%). The average age of the involved patients was approximately 74.8±16.67 years, and the range of ages in the dataset is in 1-97 years, with a gender distribution of 637 (58.28%) males and 470 (42.72%) females.
Conclusions: Our findings reveal that pus serves as a significant reservoir for ESKAPE pathogens, with *Staphylococcus aureus* and *Pseudomonas aeruginosa* as predominant organisms.

Keywords: ESKAPE pathogens, Nosocomial pathogens, Multidrug resistance, Anti-microbial resistance

INTRODUCTION

'ESKAPE' is the acronym for six pathogens of current interest in the landscape of infectious diseases. Which include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species. Their acronym aptly captures their remarkable capacity to 'escape' the effects of antimicrobial treatments, posing a grave threat to patient outcomes and public health globally. The inefficiency of antimicrobials against these pathogens is due to various resistance mechanisms such as drug inactivation, modification of drug binding sites/targets, changes in cell permeability and/or mutation.[1] These pathogens, notorious for their ability to cause nosocomial infections, have become increasingly adept at circumventing the arsenal of antibiotics leading to a surge in multidrug-resistant strains that defy conventional therapeutic interventions.[2] The major concern related to infection with such pathogens are bad

patient outcomes such as longer hospital stay, higher morbidity and mortality associated with antibiotic-resistant bacteria's.[3,4] One of the main factors behind the development of antimicrobial-resistance is overuse and misuse of antibacterial agents.[5,6] Multidrug resistance (MDR) is extending continuously across the globe and it is a challenge in treating infections, making it necessary to use the reserve antibiotics which can have higher cost-to-benefit ratios and a decreased security profile.[7,8] Therefore rationalized use of antibiotics is pivotal to curb antibiotic resistance.

Understanding the distribution patterns of ESKAPE pathogens across diverse clinical samples is crucial for elucidating their epidemiology and devising targeted control measures. Furthermore, unraveling the intricate web of antimicrobial resistance exhibited by these pathogens is essential for guiding empirical treatment strategies and preserving the efficacy of existing antimicrobial agents. However, despite the urgency of this task, comprehensive data on the prevalence and resistance profiles of ESKAPE pathogens in various clinical settings remain limited.

This research aims to address the knowledge gap by systematically deciphering the distribution of ESKAPE pathogens in a wide array of clinical samples obtained from both community and healthcare settings. We aim to delineate the prevalence, diversity, and resistance patterns of ESKAPE pathogens across different patient populations. The study aims to find out the dynamics and characteristics of infections caused by ESKAPE pathogens and its drug resistance.

METHODS

Study place

The study was conducted in Dr. Moopen's Medical College, Wayanad.

Study duration

The period of study was of 6 months starting from April to October 2023.

Sample collection

Samples are meticulously collected from patients admitted to intensive care unit (ICU), general wards, and the emergency department. This broad sampling strategy ensures representation across different patient demographics and clinical conditions, enhancing the generalizability of the findings.

Study design

This study was formulated to assess the prevalence of ESKAPE pathogen in a hospital setting over a specific time frame. Since this study was purely based on data analysis on a retrospective basis and there were no patient involvement or sample processing, direct consent collections were excluded.

Sampling method

Data collection based on samples with isolates of ESKAPE pathogens from pus, urine and sputum. Only patients with confirmed infections caused by ESKAPE pathogen were considered for the study. Data collection through patient registers of microbiology laboratory at the hospital.

Sample size calculation

The minimum sample size required for the study comes to 483. This was calculated based on the prevalence of ESKAPE pathogens, as evidenced by prior research, prevalence of 87.4% seen in urinary tract infections among Jordanian patients with 95% accuracy and with 3% precision.

A total of 6492 clinical samples were studied to isolate ESKAPE pathogens. Which included urine, sputum and pus samples. This determination is based on a thorough calculation considering the prevalence of ESKAPE pathogens, as evidenced by prior research. Specifically, the prevalence of ESKAPE pathogens in urinary tract infections (UTIs) among Jordanian patients, serves as a key reference point. Each patient's demographic information, including age and gender, is recorded alongside details regarding the type of sample collected, the specific organism isolated, and the corresponding antibiotic resistance profile.[9]

Statistical analysis

The data were collected and stored in MS excel. Analysis was carried out using SPSS 26.0 version. All continuous variables were expressed in mean, median and deviations. Categorical variables were expressed using frequency and percentage.

Ethical approval

Confidentiality was maintained by securely storing and anonymizing data. Ethical perspectives and safety considerations: Confidentiality and privacy was ensured in handling patient data by conducting the data collection within the labs. Also adhered to ethical guidelines for research involving human subjects.

RESULTS

A total of 6,492 samples were studies which include urine, pus and sputum. The study was aiming to identify the prevalence of ESKAPE pathogens from these samples. Most of the ESKAPE isolates (49%) were identified from pus samples followed by sputum and urine (Table 1).

The prevalence of ESKAPE pathogens noted was different in each sample. The most predominant bacteria were found to be *Staphylococcus aureus* (31.39%) in pus whereas *Klebsiella pneumoniae* in urine (40.10%) and sputum samples (40.48%) as shown in table 1. *Klebsiella pneumoniae* was observed to be prevalent in respiratory sample (38.21%) followed by pus (32.02%) and urine (29.77%). *Acinetobacter baumannii* was most prevalent in respiratory sample (43.14%) followed by pus (40.19%) and then urine (16.67%). *Pseudomonas aeruginosa* was most prevalent in pus (45.52%) then in respiratory sample (39.02%) and urine (15.46%). Enterobacter was observed most in pus (54%) followed by urine (25%) and respiratory sample (21%) (Table 1).

Table 1. Distribution of ESKAPE isolates in each clinical sample.

Acronym	Bacterial Family/Genus/Species	Pus (%)	Urine (%)	Respiratory sample (%)
E	*Enterococcus spp.*	77 (13.27%)	66 (24.90%)	6 (1.78)
S	*Staphylococcus aureus*	182 (31.39%)	13 (4.90%)	33 (9.83)
K	*Klebsiella pneumonia*	114 (19.67%)	106 (40.10%)	136 (40.48)
A	*Acinetobacter baumannii*	41 (7.06%)	17 (6.41%)	44 (13.09)
P	*Pseudomonas aeruginosa*	112 (19.30%)	38 (14.30%)	96 (28.58)
E	*Enterobacter spp.*	54 (9.31%)	25 (9.42%)	21 (6.25)
Total	1181	580 (100%)	265 (100%)	336 (100)

Table 2: Antibiotic resistance levels of ESKAPE isolates during the study period.

Bacterialfamily/Genus/Species	Isolates	ESBL	MBL	MDR	VRE*	XDR*	MRSA
Enterococcus spp.	149	-	-	-	0%	-	-
Staphylococcus aureus	228	-	-	-	-	-	92 (40.35)
Klebsiella pneumoniae	356	23 (6.46%)	0 (0%)	46 (12.92%)	-	0%	-
Acinetobacter baumannii	102	1 (0.98%)	7 (6.86%)	39 (38.23%)	-	0%	-
Pseudomonas aeruginosa	246	5 (2.03%)	5 (2.03%)	13 (5.28%)	-	0%	-
Enterobacter spp.	100	5 (5%)	0 (0%)	7 (7%)	-	0%	-
Total	1181	34 (2.87%)	12 (1.01%)	105 (8.89%)		0%	92 (7.79)

Table 3: Epidemiology and distribution of ESKAPE pathogens during the study period.

Gram stain	Bacterial Family/Genus/Species	Frequency (%)
Gram-positive n=377 (31.95%)	*Enterococcus spp.*	149 (12.61)
	Staphylococcus aureus	228 (19.34)
Gram-negative n=804 (68.05%)	*Klebsiella pneumoniae*	356 (30.14)
	Acinetobacter baumannii	102 (8.63)
	Pseudomonas aeruginosa	246 (20.82)
	Enterobacter spp.	100 (8.46)
Total		1181 (100)

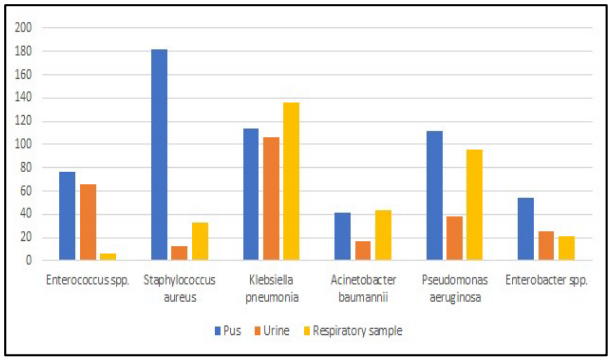

Figure 1: Drug resistance by ESKAPE pathogens.

In pus, the prevalent isolate noted from highest to lowest is *Staphylococcus aureus* (31.39%), *Klebsiella pneumoniae* (19.67%), *Pseudomonas aeruginosa* (19.30%), Enterococcus spp. (13.27%), Enterobacter spp. (9.31%) and *Acinetobacter baumannii* (7.06%) respectively. In Urine, *Klebsiella pneumoniae* (40.10%), *Enterococcus spp.* (24.90%), *Pseudomonas aeruginosa* (14.30%), *Enterobacter spp.* (9.42%), *Acinetobacter baumannii* (6.41%) and *Staphylococcus aureus* (4.90%). In sputum samples, *Klebsiella pneumoniae* (40.48%), *Pseudomonas aeruginosa* (28.58%), *Acinetobacter baumannii* (13.09%), *Staphylococcus aureus* (9.83%), *Enterobacter spp.* (6.25%) and *Enterococcus spp.* (1.78%).

Drug resistance by ESKAPE pathogens

A total of 149 Enterococcus isolates were tested for antibiotic susceptibility and was sensitive towards almost all commonly used antibiotics. Among 228 Staphylococcus aureus isolates, 92 (40.35%) were MRSA, indicating resistance to methicillin. Out of 356 *Klebsiella pneumoniae* isolates, 23 (6.46%) showed resistance to ESBL, 46 (12.92%) were MDR. Among 102 *Acinetobacter baumannii* isolates, 1 (0.98%) showed resistance to ESBL, 7 (6.86%) MBL, and 39 (38.23%) MDR. Out of 246 *Pseudomonas aeruginosa* isolates, 5 (2.03%) exhibited resistance to ESBL and MBL, and 13 (5.28%) were MDR. Among 100 Enterobacter isolates, 5 (5%) showed resistance to ESBL, and 7 (7%) MDR. This data underscores the importance of monitoring antibiotic resistance patterns among various bacterial species to guide appropriate treatment strategies and prevent the spread of resistant strains. The overall resistance level of ESKAPE isolates is summarized in Table 2.

Distribution of bacterial isolates

During the study period, total of n=6,492 Individual bacteria isolates were recovered from clinical specimens such as sputum (1473), urine (3710) and pus (1309) out of which, isolates n=1181 were ESKAPE pathogen. The study observed an overall dominance of gram-negative bacteria (shown in Table 3). *Klebsiella pneumoniae* was the most frequently isolated bacteria, n=356 (30%), followed by *Pseudomonas aeruginosa* n=246, (20.8%), *Staphylococcus aureus* n=228, (19.34%), and *Enterococcus spp.* n=149, (12.61%).

Demographic and clinical features of the study population

The average age of the involved patients was approximately 74.8±16.67 years, and the range of ages in the dataset is in 1-97 years, with a gender distribution of 637 (58.28%) males and 470 (42.72%) females.

DISCUSSION

In our study, total of n=6,492 Individual bacteria isolates were recovered from clinical specimens such as sputum (1473), urine (3710) and pus (1309) out of which n=1181 were ESKAPE pathogen. A study conducted by Benko R et al, from Hungary in the year 2020 studied a total of 4974 (72.22%) ESKAPE pathogen out of n=6887 samples.10 Where another study conducted by Arbune M et al, studied a total of n 4164 ESKAPE pathogens out of n=4293 clinical samples.[11]

The prevalence of ESKAPE pathogens noted was different in each sample. The most predominant bacteria were found to be *Staphylococcus aureus* (31.39%) in pus whereas *Klebsiella pneumoniae* in urine (40.10%) and sputum samples (40.48%) on contrary to study conducted in Hungary where it showed a marked prevalence of *Enterobacter spp.* which accounts for 243 (49.19%) out of 494 pus samples, 1672 (70.1%) out of 2385 urine sample and </=250 (42.51%) out of 588 sputum samples.[10] While taking pus into analysis *Staphylococcus aureus* occupies the larger proportion 182 (31.39%) whereas *Acinetobacter baumannii* was the least predominant 41 (7.06%), On contrary to study conducted by Benko R et al it showed a marked prevalence of Enterobacter spp. which accounts for 243 (49.19%) out of 494 pus samples but the least predominant was *Acinetobacter baumannii* 5 (16.1%) which almost corresponds to our study. While taking urine into consideration, *Klebsiella pneumonia* accounts for the most in urine 106 (40.10%) and *Staphylococcus aureus* was the least 13 (4.90%), But in a study conducted by Benko R in the year 2020, The results showed a marked prevalence of *Enterobacter spp*. viz. 1672 (70.1%) out of 2385 urine samples and the least predominant was *Acinetobacter baumannii* (19.4%).On detailed analysis of sputum samples, *Klebsiella pneumoniae* (40.48%) was most predominant whereas *Enterococcus spp* (1.78%) was the least predominant, contrary to our results regarding the prevalence of ESKAPE pathogens Pandey R et al reported the marked prevalence of Pseudomonas aeruginosa n=32 (32.98%) out of 97 sputum samples whereas the least predominant was *Enterococcus faecium* n=1 (0.01%).[11]

A total of 149, Enterococcus isolates were tested for antibiotic susceptibility and was sensitive towards almost all commonly used antibiotics. A study conducted in Nepal documented that n=5 (20%) out of total n=25 showed resistance to vancomycin. Among 228 Staphylococcus aureus isolates, 92 (40.35%) were MRSA, indicating resistance to methicillin, contrary to our results regarding the resistance of *Staphylococcus aureus*, Benko R et al, reported a methicillin resistance of 16.9% with no identifiable temporal trends. Whereas a study conducted by Pandey et al, showed that 57.6% of S. aureus were methicillin-resistant. Out of 356 *Klebsiella pneumoniae* isolates, 23 (6.46%) showed resistance to ESBL, 46 (12.92%) were MDR. But a study showed that among *Klebsiella pneumonia* isolates, n=24 (16.1%) had resistance to ESBL, n=48 (32.2%) was MDR, N=19(12.8%) were XDR and n=12 (8.1%) was MBL. Among 102 *Acinetobacter baumannii* isolates, 1 (0.98%) showed resistance to ESBL, 7 (6.86%) MBL, and 39 (38.23%) MDR. Whereas in a study conducted in Nepal in the year 2021 showed that among *Acinetobacter baumannii* isolates n=12 (30.76%) was MDR, n=12 (30.76%) was XDR, n=4 (10.3%) was ESBL resistant and n=4 (10.3%) was MBL resistant. Out of 246 *Pseudomonas aeruginosa* isolates, 5 (2.03%) exhibited resistance to ESBL and MBL, and 13 (5.28%) were MDR. But the study showed that among *Pseudomonas aeruginosa* isolates, n=12 (14.3%) was MDR, n=6 (7.1%) was XDR, n=9 (10.7%) was ESBL resistant and n=7(8.3%) was MBL resistant. Among 100 Enterobacter isolates, 5 (5%) showed resistance to ESBL, and 7 (7%) MDR, Contrary to our results regarding the multidrug

resistance Pandey et al reported a high prevalence of MDR among Enterobacter.[4,11]

CONCLUSION

In conclusion, our research provides valuable insights into the distribution of ESKAPE pathogens across various clinical samples and their patterns of antimicrobial resistance, with a particular focus on pus. Our findings reveal that pus serve as a significant reservoir for ESKAPE pathogens, with Staphylococcus aureus and *Pseudomonas aeruginosa* emerging as predominant organisms isolated from this sample type.

The prevalence of *Staphylococcus aureus* and Pseudomonas aeruginosa in pus underscores the clinical relevance of these pathogens in wound infections and other exudative conditions. The high isolation rates of these organisms highlight the importance of targeted surveillance and infection control measures to mitigate their spread within healthcare settings. Moreover, our study elucidates the antimicrobial resistance patterns of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, providing critical insights for guiding empirical treatment strategies. While our study focused on pus, future research should explore the distribution and antimicrobial resistance profiles of ESKAPE pathogens across a broader range of clinical samples. Additionally, longitudinal studies are warranted to monitor changes in pathogen prevalence and antimicrobial resistance over time, thereby informing targeted interventions to mitigate the spread of multidrug-resistant microbes. In conclusion, our research contributes to the growing body of knowledge surrounding ESKAPE pathogens and underscores the importance of continued surveillance and research efforts to combat antimicrobial resistance effectively.

However, this study has several limitations, since it is a retrospective cross-sectional design the data collection was purely based on documentation and the study was conducted collecting the data from a single hospital which may not capture the actual spectrum of prevalence in an area.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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