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Original research

ANTIBIOTIC RESISTANCE CHARACTERISTICS OF THE *ESKAPEE* PATHOGENS GROUP ISOLATED AT HUE UNIVERSITY OF MEDICINE AND PHARMACY HOSPITAL IN 2024

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ABSTRACT

Background: *The current state of infections in general and hospital-acquired infections is increasingly rising, not only in Vietnam but also worldwide. This study aims to evaluate the antibiotic resistance characteristics of ESKAPEE pathogens isolated from clinical samples at Hue University of Medicine and Pharmacy Hospital in 2024.*

Methods: *We designed the study using a cross-sectional descriptive method and a convenience sample approach. The study was conducted at the Department of Microbiology, Hue University of Medicine and Pharmacy Hospital from January 2024 to December 2024.*

Results: *Among 1001 ESKAPEE group-isolated pathogens, Escherichia coli and Staphylococcus aureus were both accounted for with a high proportion (33.67%; 23.58%). In addition, these two bacteria also exhibited the high rate of resistance type for methicillin (known as MRSA) and produced extended-spectrum beta-lactamase (known as ESBL). 100% of the Staphylococcus aureus showed their resistance ability to penicillins group, while the Gram negative bacteria showed high resistance to ampicillin (90.21%) and cefazolin (71.21%). 59.74% of the isolated strains from this research belonged to multidrug-resistant strains, in which Escherichia coli was accounted for with the highest rate (78.93%).*

Conclusions: *This study revealed a concerningly high rate of antimicrobial resistance among ESKAPEE-isolated pathogens at Hue University Medicine and Pharmacy Hospital in 2024. Immediate action is necessary to implement an antibiotic stewardship program aimed at reducing the current levels of antibiotic resistance and preventing further complications.*

Keywords: *ESKAPEE; MRSA; ESBL; multidrug resistant bacteria.*

I. INTRODUCTION

The current state of infections is increasingly rising, not only in Vietnam but also worldwide [1]. Among them, hospital-acquired infections (HAIs) are one of the top challenges and concerns. HAIs have been and continue to cause severe consequences such as prolonged hospital stays, increased healthcare costs, and higher mortality rates [2].

The ESKAPEE group of pathogens is an extension of the ESKAPE group, which is an acronym for six highly virulent bacteria that are resistant to many groups of antibiotics, including *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter* spp., and *Escherichia coli.* With various pathogenic mechanisms as well as the ability to evade different antibiotic effects, the existence of these pathogens is the cause of many severe inpatient cases or special hospital-acquired infections in patients with immunocompromised conditions or high-risk diseases [3]. Due to the impact of infections caused by multidrug-resistant organisms, the World Health Organization (WHO) created the bacterial priority

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pathogens list (bacterial priority pathogens list-BPPL) in February 2017, which was recently updated in 2024 [4]. Because of their multidrug-resistant status and heightened resistance to last-resorted medications, the *ESKAPEE* infections are classified as organisms of vital relevance, necessitating the immediate development of novel antibiotics [5].

Therefore, identifying the prevalence of the *ESKAPEE* group of pathogens isolated from patients visiting or receiving treatment at the hospital contributes significantly to the control of HAIs, while also providing timely warnings to prevent the spread of infections and the risk of antibiotic resistance among the strains within that group. The existence of the *ESKAPEE* group has also been reported in various studies worldwide in infections isolated from different hospital units [2, 6, 7] and also in the environment [8]. In Vietnam, researchers mainly focus on the resistance mechanisms of individual multidrug-resistant organism strains and the *ESKAPE* [9], but the reporting on the infection situation and especially the resistance capabilities to antibiotic groups of *ESKAPEE* pathogens is still very limited and almost lacks complete statistics. Therefore, we conducted this study with the aim of evaluating the antibiotic resistance characteristics of *ESKAPEE* group strains isolated from various clinical samples of outpatients and inpatients at Hue University of Medicine and Pharmacy (HueUMP) Hospital in 2024.

II. MATERIALS AND METHODS

2.1. Research subjects

Inclusion criteria: All strains of bacteria belonging to the *ESKAPEE* group were cultured, isolated, and identified from clinical samples of patients visiting and receiving treatment at HueUMP Hospital. The bacterial strains of the same type isolated multiple times from the same patient are only recorded from the first isolation.

Exclusion criteria: Bacterial strains isolated for the second time identified as the same type/strain as the first time in the same patient; cases without sufficient necessary information for the study are also excluded from the study.

2.2. Methods

A cross-sectional descriptive study with a convenience sampling was conducted at the Department of Microbiology, HueUMP Hospital, from January 2024 to December 2024.

2.3. Data collection methods

Retrieval of electronic data stored at the Department of Microbiology from all specimens which had isolated pathogens and indications for performing antibiotic susceptibility from patients who visited and received treatment at HueUMP Hospital during the study period, meeting the inclusion and exclusion criteria.

All types of different specimens (pus, wound exudate, blood, sputum, urine, various body fluids...) indicated for culture from clinical departments were cultured and identified according to the standard procedures of the Department of Microbiology, HueUMP Hospital. Tests for detecting the phenotypic resistance of bacteria producing extended-spectrum β-lactamase (ESBL) and methicillin-resistant *Staphylococcus aureus* (MRSA) were conducted according to the recommendations of CLSI (Clinical and Laboratory Standards Institute M100 [10]. Additionally, the disk diffusion method (Kirby-Bauer method) was also used to test the antibiotic susceptibility of different groups to see how resistant they were to them. This procedure was carried out according to the standard procedure issued by the Ministry of Health in 2017, with adjustments made as appropriate [11]. The results of bacterial sensitivity to antibiotics were evaluated by comparing the diameter of the inhibition zone measured in millimeters (mm) with the breakpoint values in the CLSI M100 - 2023 standard documents or according to EUCAST (European Committee for Antimicrobial Susceptibility Testing) for each type of antibiotic to determine sensitivity (S = Susceptible), intermediate (I = Intermediate) and resistance (R = Resistant) [10].

In addition, the MDR (multidrug-resistant) bacterial strains, defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories were also reported among the isolated *ESKAPEE* group [12].

2.4. Data processing and analysis methods

All data were statistically processed using Excel software, and then the data were encoded as variables, and the collected data were processed using SPSS 20.0 statistical software.

III. RESULTS

3.1. Participants' characteristics

Among the 823 patients who participated in the study, the majority were male, accounting for 52.98%. The median age of the participants was 56 years, with the age group of 19 to 59 years representing the highest percentage at 47.26%. The number of specimens was highly collected from the department of Orthopedics and Trauma-Thoracic Surgery with 275 samples (27.47%), followed by the Urology Outpatient Clinic with 144 samples (14.38%).

2.2. The proportion of *ESKAPEE* pathogens group isolated from different samples

A total of 1001 *ESKAPEE* bacterial pathogens were isolated from 823 patient samples. In detail, Gram-negative accounted for a high proportion of 68.23% (683 strains), and Gram-positive accounted for 31.77% (318 strains). In the Gram-negative group, *Escherichia coli (E.coli)* had the highest proportion of 33.67%, followed by *Pseudomonas aeruginosa (P.aeruginosa)* 16.68%, *Klebsiella pneumoniae (K.pneumoniae)* 8.79%, *Enterobacter* spp. 6.59%, and the lowest was *Acinetobacter baumannii (A. baumannii)* 2.50%. In the Gram-positive group, *Staphylococcus aureus (S.aureus)* had a high proportion of 23.58%, and *Enterococcus* spp. made up 8.19% (Figure 1). The most common sources of the specimen were wound swabs/pus (56.04%) and urine (28.87%), while blood and respiratory specimens had the lower percentages, 5.19% and 3.15%, respectively.

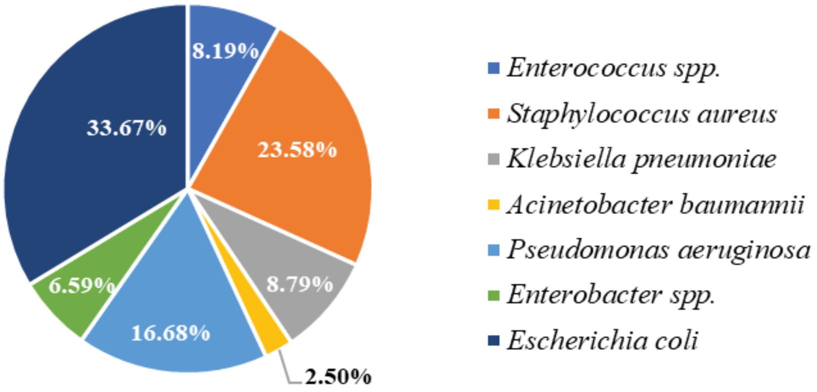

Figure 1: Proportions of ESKAPEE group isolated bacteria

2.3. Prevalence of MRSA and ESBL producing isolates among *ESKAPEE* group

From a total of 236 *S. aureus* isolates, there were 163 strains which tested positive for MRSA accounting for 69.07% (Table 1). Out of a total of 491 bacterial isolates, 209 (42.57%) were found to produce Extended-Spectrum Beta-Lactamase (ESBL). Among these, *Escherichia coli* exhibited the highest rate of ESBL production at 50.15%, followed by *Klebsiella pneumoniae* at 34.09%, and *Enterobacter* spp. at 15.15% (Table 2).

Table 1: Percentage of Methicillin-resistant *S. aureus*

Resistance type / Bacterial strains	N	MRSA (+)		MRSA (-)	
		n	%	n	%
Staphylococcus aureus	236	163	69.07	73	30.93

Table 2: Percentage of bacteria produced ESBL

Resistance type / Bacterial strains	N	ESBL(+)		ESBL(-)	
		n	**%**	**n**	**%**
Escherichia coli	337	169	50.15	168	49.85
Klebsiella pneumoniae	88	30	34.09	58	65.91
Enterobacter spp.	66	10	15.15	56	84.85
Total	491	209	42.57	282	57.43

2.4. Drug resistance patterns of *ESKAPEE* group isolates to different classes of antibiotics

Antibiotic resistance classes of the isolated pathogen of nosocomial origin are shown in Table 3. In the Gram-positive group. nearly two-thirds of *S. aureus* isolates were resistant to methicillin/cefoxitin/oxacillin (68.64%). and Clindamycin (69.49%), respectively, and all of *S. aureus* isolates were resistant against penicillin (Table 1). At the same time, *Enterococcus* spp. had the lower proportion of resistance, with gentamicin exhibiting the highest resistance rate at 51.22%. Regarding the Gram-negative group, antibiotic resistance was notably higher, that 304 (90.21%) *E.coli* strains resistant to Ampicillin, followed by *Enterobacter* spp with 71.24%. Besides, Cefazolin also demonstrated the high resistance rates among *Enterobacter* species, *E. coli*, and *Klebsiella pneumoniae*, with resistance rates of 71.21%, 70.03%, and 50.00%, respectively.

Table 3: Percentage of antimicrobial-resistant bacterial isolates for classes of antimicrobials

Antibiotic groups	**Antibiotic**	***Enterococcus* spp.** **n = 82 (%)**	***Staphylococcus aureus*** **n = 236 (%)**	***Klebsiella pneumoniae*** **n = 88 (%)**	***Acinetobacter baumannii*** **n = 25 (%)**	***Pseudomonas aeruginosa*** **n = 167 (%)**	***Enterobacter* spp.** **n = 66 (%)**	***Escherichia coli*** **n = 337 (%)**
Penicillins	Penicillin	23(28.05)	234(99.15)	-	-	-	-	-
	Oxacillin		162(68.64)	-	-	-	-	-
	Ampicillin	16(19.51)	-	-	-	-	47(71.21)	304(90.21)
Beta-lactam combination	Amoxicillin-clavulanic acid	-	-	14(15.91)	-	-	18(27.27)	52(15.43)
	Ampicillin-sulbactam	-	-	32(36.36)	3(12.00)	-	25(37.88)	80(23.74)
	Piperacillin-tazobactam	-	-	19(21.59)	3(12.00)	52(31.14)	3(4.55)	23(6.82)
Glycopeptides	Vancomycin	3(3.66)	-	-	-	-	-	-
Cephems	Cefazolin	-	-	44(50.00)	-	-	47(71.21)	236(70.03)
	Cefuroxime	-	-	31(35.23)	-	-	22(33.33)	157(46.59)
	Cefoxitin	-	162(68.64)	-	-	-	-	-
	Ceftriaxone	-	-	-	2(8.00)	-	5(7.58)	18(5.34)
	Ceftazidime	-	-	28(31.82)	3(12.00)	54(32.34)	9(13.64)	77(22.85)
	Cefotaxime	-	-	42(47.73)	4(16.00)	-	19(28.79)	187(55.49)
	Cefepime	-	-	25(28.41)	3(12.00)	48(28.74)	8(12.12)	66(19.58)
Carbapenems	Ertapenem	-	-	16(18.18)	-	-	5(7.58)	12(3.56)
	Imipenem	-	-	7(7.95)	4(16.00)	53(31.74)	10(15.15)	2(0.59)
	Meropenem	-	-	12(13.64)	5(20.00)	57(34.13)	7(10.61)	7(2.08)

Antibiotic groups	**Antibiotic**	***Enterococcus* spp. n = 82 (%)**	***Staphylococcus aureus* n = 236 (%)**	***Klebsiella pneumoniae* n = 88 (%)**	***Acinetobacter baumannii* n = 25 (%)**	***Pseudomonas aeruginosa* n = 167 (%)**	***Enterobacter* spp. n = 66 (%)**	***Escherichia coli* n = 337 (%)**
Oxazolidinoes	Linezolid	6(7.32)	-	-	-	-	-	-
Aminoglycosides	Gentamicin	42(51.22)	-	27(30.68)	5(20.00)	49(29.34)	13(19.70)	116(34.42)
	Tobramycin	-	-	6(6.82)	1(4.00)	17(10.18)	3(4.55)	39(11.57)
	Amikacin	-	-	-	3(12.00)	36(21.56)	2(3.03)	13(3.86)
Macrolides	Erythromycin	-	159 (67.37)	-	-	-	-	-
Tetracyclines	Tetracycline	26(31.71)	101(42.80)	-	-	-	-	-
BởFluoroquinolones	Ciprofloxacin	23(28.05)	-	33(37.50)	4(16.00)	66(39.52)	9(13.64)	188(55.79)
	Norfloxacin	5(6.10)	-	-	-	43(25.75)	-	-
	Levofloxacin	21(25.61)	-	28(31.82)	3(12.00)	59(35.33)	8(12.12)	197(58.46)
Licosamides	Clindamycin	-	164(69.49)	-	-	-	-	-
Folic Acid synthesis inhibitors	Trimethoprim-sulfamethosazole	-	44(18.64)	34(38.64)	6(24.00)	-	15(22.73)	221(65.58)
Fosfomycins	Fosfomycin	-	-	-	-	-	-	1(0.30)
Nitrofurans	Nitrofurantoin	2(2.44)	-	6(6.82)	-	-	5(7.58)	1(0.30)

2.5. Classification of isolates based on their drug resistance pattern

In 1001 isolated *ESKAPEE* group bacteria strains, multidrug-resistant (MDR) strains accounted for a high rate of 59.74%. *E.coli* had the highest MDR rate (78.93%), followed by *S. aureus* and *Enterobacter* spp. with the MDR rates of 69.92% and 57.58%, respectively (Table 4).

Table 4: Percentage of multidrug-resistant organism

Organisms	N	Non-MDR(%)	MDR(%)
Enterococcus spp.	82	60 (73.18%)	22 (26.83%)
Staphylococcus aureus	236	71 (30.08%)	165 (69.92%)
Klebsiella pneumoniae	88	48 (54.55%)	40 (45.45%)
Acinetobacter baumannii	25	20 (80.00%)	5(20.00%)
Pseudomonas aeruginosa	167	105 (62.87%)	62(37.13%)
Enterobacter spp.	66	28 (42.42%)	38 (57.58%)
Escherichia coli	337	71 (21.07%)	266 (78.93%)
Total	1001	403 (40.26%)	598 (59.74%)

III. DISCUSSION

The rise of antimicrobial resistance in *ESKAPE* and *ESKAPEE* pathogens has become a significant concern worldwide in managing infectious diseases, emphasizing the important impact of these microorganisms. Recently, some reports have described resistance patterns as an emerging part of a more significant crisis, and our findings of this study indicated the emergence of alarming patterns of resistance among *ESKAPEE* pathogens in hospitalized patients collected at HueUMP Hospital.

The predominance of Gram-negative bacteria in our study aligned with findings from other regions. For instance, E. coli (33.67%) was the most frequent isolate bacterium among *ESKAPEE* pathogens, followed by S.aureus (23.58%), and *P.aeruginosa* (16.68%). Conversely, the lowest rate of one was *A.baumannii* (2.50%). Our result was similar to research in Greece, with the proportion of *E.coli* at 33% [6], and different from the results in Palestine where *A.baumannii* were more prominent at 40.8% [13]. These etiologies highlight the need for localized epidemiological studies to accurately assess the prevalence and distribution of Gram-negative bacteria within specific healthcare environments. Such information is essential for shaping infection control measures and informing empirical antibiotic treatment.

The high prevalence of *MRSA* was once a critical antimicrobial resistance issue for *S. aureus* all over the world, in our study, that rate made up 69.07%. With the same result, a report at Danang Oncology Hospital showed that 76.1% of patients infected with *S.aureus* from collected samples period (January 2019 to July 2021) were *MRSA* [14]. In many countries, the proportion of *MRSA* surpassed 20%, with some cases reaching as high as 80% [13]. The WHO's report on Egypt was based on a single study that analyzed 122 isolates, finding an *MRSA* prevalence of 46% [15]. Besides, clindamycin and erythromycin also had high resistance rates at 69.49% and 67.37%, respectively. This led to *S.aureus* being a pathogen in the *ESKAPEE* group, having the highest proportion resistance (69.92%). However, in our study, we had not recorded any cases of *S. aureus* that are resistant to linezolid, which could be a suitable choice for treatment. Another pathogen in the Gram-positive group is *Enterococcus* spp., with the percentage of linezolid and vancomycin still low resistance at 7.32% and 3.66%, respectively. Linezolid serves as one of the final lines of defense against Gram-positive bacteria, particularly in cases where staphylococci are resistant to multiple antibiotics. The widespread use of antimicrobial agents in clinical treatment has led to the emergence and global spread of antibiotic-resistant enterococci, particularly multi-drug resistant (MDR) isolates such as Vancomycin-resistant Enterococci (VRE) and Linezolid-resistant Enterococci (LRE) [16].

In terms of the Gram-negative group, the emergence of ESBL-producing Gram-negative rods has become a rising concern in the developing world [17]. In this study, the research indicated that 42.57% of the 491 bacterial isolates tested were found to produce Extended-Spectrum Beta-Lactamases. The rate of ESBL production among the isolates was dominated by *E.coli* (50.15%), followed by *K.pneumoniae* (34.09%), and *Enterobacter* spp. (15.15%). This finding emphasized the significant presence of ESBL-producing organisms in the studied population, with *E.coli* emerging as the most prolific producer of ESBLs. The high percentage of ESBL production among these isolates was concerning, as it suggested an increasing trend of antimicrobial resistance, particularly to beta-lactam antibiotics. This finding was consistent with previous studies. For instance, the study conducted in seven hospitals across Ho Chi Minh city found that the positive rate for ESBL of *E.coli* and *K.pneumoniae* were 58% and 23.60%, respectively [18]. Specially, Vietnam's prevalence of ESBL-producing Enterobacterales was notably high (55.10%), surpassing those in neighboring countries such as Thailand (38.40%) and Cambodia (40.60%) [19]. Likewise in another country, the research in Saudi Arabia (2023) showed that *E.coli* was the commonest ESBL producer at 51.4%, followed by *K.pneumoniae* at 27% [20]. Furthermore, *E.coli* was also the group of bacteria that had the highest percentage of MDR at 78.93%. *E.coli* exhibited high resistance rates to ampicillin (90.2%), cefazolin (70.03%), cefotaxime (55.49%), ciprofloxacin (55.79%), levofloxacin (58.46%) and trimethoprim-sulfamethosazole (65.58%). On the other hand, the *E.coli* strains isolated from patients visiting and receiving treatment in our study were highly sensitive (nearly 100%) to the two antibiotic groups, fosfomycin and nitrofurantoin. In contrast, the research in China collected data from 2014 to 2021 displaying a notable upward trend in resistance to fosfomycin from 0.1% to 3.57%[16]. The next, MDR *P. aeruginosa*, poses an increasing global threat of nosocomial infections. In this study, we found a relatively high resistance rate of *P.aeruginosa* strains to ceftazidime combined with avibactam (34.13%) and the carbapenem group, including imipenem (31.74%) and meropenem (34.13%), higher than the research in China with the rate under 30% [16]. The another group of Gram-negative bacteria, such as *A.baumannii* in this study had the a lower rate of MDR (5%) than others group; different from a research from China that presented the remarkable proportion reaching approximately 45% [16].

Therefore, the widespread antibiotic resistance in both clinical and community settings in Vietnam is an alarming issue, and the government should take action to tackle this challenge. First, we should enhance surveillance to control resistant strains, implement antibiotic management programs to regulate usage and strengthen infection control measures like hygiene protocols and patient isolation. Second, promoting research into alternative therapies is crucial to combat resistant infections, while public awareness campaigns should focus on proper antibiotic use and hygiene to reduce community-acquired infections. These steps are important for controlling resistance and protecting public health.

IV. CONCLUSION

This study revealed a concerningly high rate of antimicrobial resistance. The prevalence of multidrug-resistant (MDR), extended-spectrum beta-lactamase (ESBL), and methicillin-resistant *Staphylococcus aureus* (MRSA) strains is alarming. It is crucial for all stakeholders within the health system at Hue University Medicine and Pharmacy Hospital to collaborate in addressing this significant public health issue. Immediate action is necessary to implement an antibiotic stewardship program aimed at reducing the current levels of antibiotic resistance and preventing further complications.

Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Hue University Medicine and Pharmacy Hospital.

Disclosure

The authors report no other conflicts of interest in this work.

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