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Study of the prevalence of ESKAPE pathogens and their resistance to antimicrobial drugs

A prospective microbiological study of the prevalence of ESKAPE microorganisms and antibiotic resistance rates of strains was conducted in patients of the pediatric cardiac surgery department of a highly specialized clinic in the period from 2019 to 2023. During the study, classical routine bacterial methods for identifying isolates were used, as well as the automated system for final identification and susceptibility testing Vitek 2 — Compact. The study included 3725 clinical samples, the frequency of pathogen detection was: *S.aureus* 35.2 %, *K.pneumoniae* 27.3 %, *A.baumannii* 14.5 %, *Ps.aeruginosa* 12.4 %, *Enterobacter sp.* 8.7 % and *Enterococcus faecium* 1.2 %. A significant increase in resistance was detected in MRSA from 13.7 % to 41.9 % (p = 0.041), in *K.pneumoniae*, resistance to carbapenems increased from 0 % to 8.3 % (p = 0.057), while we note a decrease in the prevalence of ESBL-producing strains of *K.pneumoniae* from 63.3 %. to 45.2 % (p=0.058), resistance to carbapenems in *P.aeruginosa* strains decreased from 64.3 % to 37.7 % (p=0.037), and in *A.baumannii* from 48.5 % to 19.1 % (p=0.039). According to the obtained results, in our pediatric cardiac surgery department, ESKAPE pathogens accounted for 64.2 %. The most common isolates were *S.aureus*, *K.pneumoniae* and *A.baumannii*, while there was a sharply increasing trend towards resistance of *K.pneumoniae* to carbapenems and MRSA. Our results showed that well-designed infection control in each hospital is necessary, including a good hygiene strategy, microbiological monitoring and in-hospital control.

Keywords: microbiological monitoring, ESKAPE microorganisms, prevalence, antibiotic resistance, pediatric cardiac surgery.

Introduction

Despite technological advances in modern microbiological laboratory diagnostics, efforts to prevent infections, and the use of last-line antibiotics, bacterial infections remain a significant concern in the postoperative period of pediatric cardiac surgery [1-2]. Several key factors contribute to an increased risk of infection, including young age, delayed sternal closure, the use of intravascular devices, and prolonged ICU stays [3–5].

Cephalosporin-class antibiotics are considered the first-line treatment for severe infections caused by Gram-negative bacteria such as *Klebsiella pneumoniae*, *Enterobacter sp*., and *Escherichia coli* (*E. coli*). However, their efficacy is increasingly compromised by the widespread production of extended-spectrum β-lactamase (ESBL) enzymes, which confer resistance to these crucial antibiotics [6].

Among carbapenem-resistant clinical strains of *Enterobacterales*, *K. pneumoniae* is the most frequent and predominant pathogen [7].

Most bacterial infections caused by *Acinetobacter baumannii* occur in hospitalized patients with prolonged exposure to the healthcare system [8]. Approximately 45 % of *A. baumannii* isolates worldwide are multidrug-resistant [9], with resistance rates exceeding 60 % in the USA [10] and 41.5 % in Latin America and the Middle East [11]. Furthermore, the detection rate of multidrug-resistant *A. baumannii* isolates is more than four times higher compared to *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* [12].

It is essential to utilize local, national, and global antimicrobial resistance surveillance data to develop effective guidelines and programs for empirical antimicrobial therapy.

These factors highlight the urgent need to study the prevalence and antibiotic resistance of bacterial pathogens, as well as to implement measures to control the spread of multidrug-resistant microorganisms.

The aim of this study was to determine the prevalence of ESKAPE microorganisms — including *Enterococcus faecium*, *Staphylococcus aureus*, and Gram-negative bacteria such as *Klebsiella pneumoniae*,

Acinetobacter baumannii, *Pseudomonas aeruginosa*, and *Enterobacter sp.* — as well as the level of antimicrobial resistance in the pediatric cardiac surgery department of a highly specialized clinic in Central Kazakhstan.

Experimental

Study Design

A prospective study on the microbial landscape and antibiotic resistance rates of bacterial strains in patients from the pediatric cardiac surgery department of a highly specialized hospital was conducted between 2019 and 2023.

Sample Collection

Clinical specimens were collected from symptomatic inpatients, neonates and children up to three years of age, who were hospitalized in the pediatric cardiac surgery unit for surgical interventions involving the heart and major vessels.

Isolates were analyzed based on the site of infection. Upper respiratory tract samples included nasopharyngeal and pharyngeal swabs, while lower respiratory tract samples consisted of tracheobronchial swabs and sputum. Other samples were obtained from surgical wounds, urine, and bloodstream infections.

All specimens were collected at the patient's bedside, transported to the microbiology laboratory under appropriate conditions, and subjected to microbiological examination within the first two hours.

Microbiological methods

Microbial cultures were grown on 5 % blood agar, MacConkey agar, Chromagar Staphylococcus aureus, Chromagar Pseudomonas aeruginosa, Chromagar Acinetobacter spp., and Oriental Chromagar (Himedia, India), with incubation at 37 °C for 18–24 hours.

Identification of Isolates

Routine microbiological identification methods included the assessment of colony morphology, hemolytic activity on selective media, Gram staining, rapid biochemical tests (coagulase, oxidase, catalase, indole), and automated identification using the Vitek 2 — Compact microbiological analyzer (bioMérieux, Marcy-l'Étoile, France).

Antibiotic Sensitivity Testing

The minimum inhibitory concentration (MIC) for antibiotic susceptibility testing was determined using an automated microdilution method on the Vitek 2 — Compact microbiological analyzer, following the manufacturer's recommendations. After 18–24 hours of incubation, the obtained strains were tested against a panel of antibiotics to determine the MIC for ESKAPE pathogens. The MIC results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [13].

Statistical Analysis

All obtained data were analyzed using Microsoft Access and Excel. Trends in prevalence and antibiotic resistance levels were assessed through linear regression based on annual data. A p-value <0.05 was considered statistically significant.

Results and Discussion

A total of 3060 isolates from 3725 clinical specimens (including upper and lower respiratory tract, wound, bloodstream, and urine samples) were included in the study from January 2019 to December 2023. Over the five years of the study, 1899 ESKAPE strains were collected, with a prevalence of 64.2 %. The ESKAPE strains were most commonly found in the upper respiratory tract (81.1 %, 1541 isolates), followed by the lower respiratory tract (12.1 %, 230 isolates), blood and wound samples (2.7 %, 52 isolates), and the urinary tract (1.2 %, 24 isolates).

The most frequently isolated pathogens from the clinical specimens were *S. aureus* (35.2 %, 670 isolates), *K. pneumoniae* (27.3 %, 528 isolates), *A. baumannii* (14.5 %, 276 isolates), *P. aeruginosa* (12.4 %, 236 isolates), *Enterobacter sp.* (8.7 %, 166 isolates), and *Enterococcus faecium* (1.2 %, 23 isolates) (Table 1).

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Table 1

Distribution of ESKAPE pathogens in clinical samples

Microorganism	Upper respiratory tract	Lower respiratory tract	Surgical wound	Urinary tract	Blood	Total ESKAPE strains
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Enterococcus faecium	1 (4,3)	3 (13,0)	6 (26,0)	11 (47,8)	2 (8,6)	23 (1,2)
Staphylococcus aureus	629 (93,8)	18 (2,6)	20 (2,9)	0	3 (0,4)	670 (35,2)
Klebsiella pneumoniae	464 (87,8)	41 (7,7)	12 (2,2)	2 (0,3)	9 (1,7)	528 (27,8)
Acinetobacter baumannii	158 (57,2)	78 (28,2)	8 (2,8)	3 (1,0)	29 (10,5)	276 (14,5)
Pseudomonas aeruginosa	135 (57,2)	83 (35,1)	4 (1,6)	7 (2,9)	7 (2,9)	236 (12,4)
Enterobacter sp.	154 (92,7)	7 (4,2)	2 (1,2)	1 (0,6)	2 (1,2)	166 (8,7)
Total isolated strains	1541 (81,4)	230 (12,1)	52 (2,7)	24 (1,2)	52 (2,7)	1899

More than 90 % of *S. aureus* and *Enterobacter sp.* strains were isolated from the upper respiratory tract, with *K. pneumoniae* accounting for 87.8 %. Non-fermenting Gram-negative microorganisms, such as *P. aeruginosa* (35.1 %, 83 isolates) and *A. baumannii* (28.2 %, 78 isolates), were the most frequent pathogens in the lower respiratory tract. The highest number of *E. faecium* isolates (47.8 %, 11 isolates) was found in the urinary tract.

Microbiological prevalence monitoring revealed an increasing trend in the detection rate of *S. aureus* from 14 % to 29.8 % ($p = 0.051$), *K. pneumoniae* from 11.3 % to 20.9 % ($p = 0.044$), and *Enterobacter sp.* from 2.7 % to 10.3 % ($p = 0.028$). At the same time, no statistically significant changes were observed in the percentage of *A. baumannii* and *P. aeruginosa* strains detected (Table 2).

Table 2

Change in prevalence trends of ESKAPE isolated microorganisms by year (2019–2023)

Microorganism	2019 n (%)	2020 n (%)	2021 n (%)	2022 n (%)	2023 n (%)	p-value[1]
Enterococcus faecium	3 (0,6)	2 (0,4)	8 (1,0)	6 (0,8)	4 (0,6)	0,631
Staphylococcus aureus	62 (14,0)	83(16,7)	200 (26,7)	147 (21,7)	178 (29,8)	0,051
Klebsiella pneumoniae	50 (11,3)	89 (17,9)	134 (17,9)	130 (19,2)	125 (20,9)	0,044
Acinetobacter baumannii	34 (7,7)	61(12,3)	68 (9,0)	71 (10,5)	42 (7,0)	0,704
Pseudomonas aeruginosa	38 (8,6)	43 (8,6)	56 (7,4)	51 (7,5)	48 (8,0)	0,245
Enterobacter sp.	12 (2,7)	7 (1,4)	29 (3,8)	56 (8,2)	62 (10,3)	0,028
Total isolates	441	495	748	676	597	

[1] Linear regression

The results of antibiotic susceptibility testing demonstrated dynamic changes in the resistance patterns of ESKAPE microorganisms, with both increasing and decreasing trends (Fig.). A significant rise in resistance was observed in *S. aureus* strains, with the prevalence of methicillin-resistant *S. aureus* (MRSA) increasing from 13.7 % to 41.9 % ($p = 0.041$). Additionally, *K. pneumoniae* strains exhibited a rise in carbapenem resistance from 0 % to 8.3 % ($p = 0.057$). During the same study period, a declining trend was observed in the prevalence of *K. pneumoniae* strains resistant to third-generation cephalosporins, decreasing from 63.3 % to 45.2 % ($p = 0.058$), as well as in carbapenem-resistant *P. aeruginosa* strains, which declined from 64.3 % to 37.7 % ($p = 0.037$), and carbapenem-resistant *A. baumannii* strains, which decreased from 48.5 % to 19.1 % ($p = 0.039$).

According to the results of resistance, there are two species (A.baumannii and P.aeruginosa), they are not susceptible to cephalosporins of the 3rd generation, and this concept applies only to K.pneumoniae. For A.baumannii and P.aeruginosa strains, it is important to determine resistance only to carbapenems, as these antibiotics are the first-line drugs of choice in the treatment of infections caused by these pathogens.

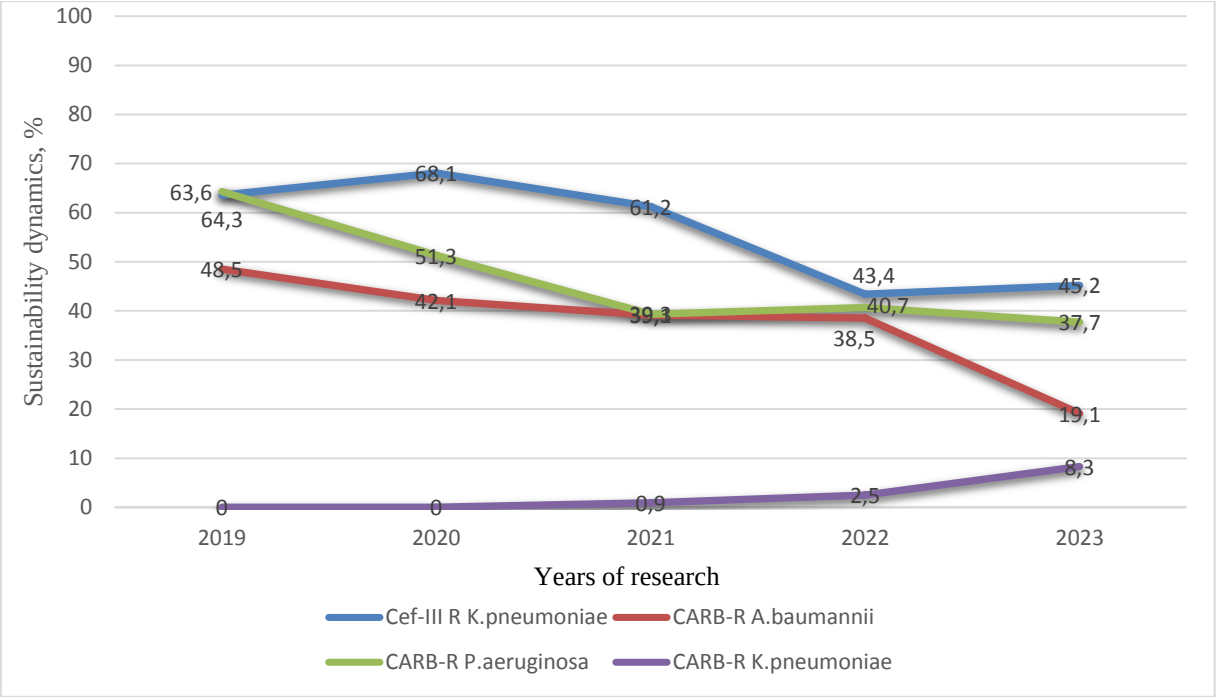

Figure. Resistance dynamics of ESKAPE Gram-negative pathogens
to third-generation cephalosporins (Cef-III R) and carbapenems (CARB-R) from 2019 to 2023.

The primary objective of this study was to assess the prevalence of ESKAPE microorganisms in clinical specimens and to evaluate their resistance to major antibacterial agents in patients from the pediatric cardiac surgery department. A total of 4114 patients hospitalized in this department were included in the study, from whom 3725 clinical specimens were collected. Among the ESKAPE pathogens, *S. aureus* was the most frequently isolated microorganism (35.2 %), followed by *K. pneumoniae* (27.8 %) and *A. baumannii* (14.5 %). *Enterobacter sp*. accounted for 8.7 % of isolates, while *E. faecium* was the least frequently detected pathogen, comprising only 1.2 % of the total isolates.

Undoubtedly, the distribution of microorganisms in patients varies between hospitals, leading to different microbiological findings [14–17]. According to previous studies, the most common pathogens in pediatric cardiac surgery patients include *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* [18-19]. Bo-Tao Ning et al. reported that the predominant pathogens were *A. baumannii* (25.6 %), *K. pneumoniae* (16.2 %), and *P. aeruginosa* (9.4 %) [19]. However, the present study on ESKAPE pathogen prevalence revealed an increasing trend in *K. pneumoniae* from 11.3 % to 20.9 % ($p = 0.044$), *S. aureus* from 14 % to 29.8 % ($p = 0.051$), and, unexpectedly, *Enterobacter sp*. from 2.7 % to 10.3 % ($p = 0.028$). In contrast to prior research by Bissenova et al. (2017) [20], no statistically significant changes were observed in the prevalence of *A. baumannii* and *P. aeruginosa*. These findings suggest a shift in the dominant pathogens within our pediatric cardiac surgery unit, with a potential emerging trend favoring *Enterobacterales*. Additionally, the majority of isolates (81.4 %) were recovered from the upper respiratory tract, likely due to the accessibility of this clinical material in the studied patient population.

The lower respiratory tract, particularly the tracheobronchial tree, is frequently contaminated with various pathogens, especially in ventilated and critically ill patients [21]. However, the correlation between bacterial colonization and the development of pulmonary infection remains unclear. A study by Johanson et al. [22] demonstrated that in 23 % of cases, bacterial colonization of clinical specimens led to pulmonary infection. Non-fermenting Gram-negative microorganisms are the predominant pathogens in the tracheobronchial tree [23], which is consistent with our findings: *A. baumannii* accounted for 28.2 % and P. aeruginosa for 35.1 % of isolates from the lower respiratory tract. Additionally, as expected, the highest proportion of *E. faecium* strains (47.8 %) was detected in the urinary tract, with no vancomycin-resistant strains identified.

Many clinical studies have reported increasing rates of ESKAPE pathogen resistance in paediatric cardiac intensive care units, making it a significant factor in nosocomial infections [24-25]. For example, Wang L.J. et al. reported that over 50 % of *P. aeruginosa* isolates (n = 126) were resistant to

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carbapenems [26]. Similarly, in our study, the rate of carbapenem resistance in *P. aeruginosa* was initially 64.3 % but showed a significant decline, reaching 37.7 % by 2023 (p = 0.037). A comparable trend was observed for *A. baumannii*, where carbapenem resistance decreased from 48.5 % in 2019 to 19.1 % in 2023 (p = 0.039). In contrast, the antimicrobial resistance patterns of *K. pneumoniae* demonstrated divergent trends over the five-year study period. While resistance to third-generation cephalosporins declined from 63.3 % to 45.2 % (p = 0.058), carbapenem resistance showed a concomitant increase from 0 % to 8.3 % (p = 0.057). Additionally, a significant rise in resistance was observed in *S. aureus*, with the prevalence of methicillin-resistant *S. aureus* (MRSA) increasing from 13.7 % to 41.9 % (p = 0.041).

The findings of this study highlight the potential for pathogen replacement, underscoring the critical need for continuous surveillance of both pathogen prevalence and antibiotic resistance rates at the hospital level and even across different departments.

It is well established that the emergence of antimicrobial resistance significantly limits therapeutic options for the management of severe infections, particularly in pediatric patients. Given the current challenges, the implementation of improved diagnostic methodologies is essential to curb the rapid dissemination of infections caused by ESKAPE microorganisms. Early and appropriate empirical antibiotic therapy — guided by clinical expertise and antimicrobial susceptibility data — is crucial for optimizing patient outcomes. Moreover, effective management of the most frequently encountered pathogens remains a key strategy in preventing the further development of multidrug resistance.

Conclusion

The findings of this study indicate that ESKAPE pathogens accounted for 64.2 % of the cases identified in the paediatric cardiac surgery department. The most frequently isolated microorganisms were *S. aureus*, *K. pneumoniae*, and *A. baumannii*, with a marked upward trend in carbapenem resistance among *K. pneumoniae* strains and an increasing detection rate of MRSA. These results underscore the critical need for stringent nosocomial infection control strategies, complemented by continuous microbiological surveillance and antimicrobial stewardship programs.

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ESKAPE патогендерінің таралуы мен микробқа қарсы тұрақтылығын зерттеу

Жоғары мамандандырылған емхананың балалар кардиохирургиясы бөлімшесінің емделушілеріне 2019-2023 жылдар аралығында ESKAPE микроорганизмдерінің таралуына және штамдардың антибиотиктерге төзімділік көрсеткіштеріне перспективалық микробиологиялық зерттеу жүргізілді. Зерттеу кезеңінде изоляттарды анықтаудың классикалық әдеттегі бактериялық әдістері, сондай-ақ Vitek 2 — Compact түпкілікті сәйкестендіру және сезімталдықты сынаудың автоматтандырылған жүйесі пайдаланылды. Зерттеуге 3725 клиникалық үлгілер енгізілді, қоздырғыштарды анықтау көрсеткіші: S.aureus 35,2 %, K.pneumoniae 27,3 %, A.baumannii 14,5 %, Ps.aeruginosa 12,4 %, Enterobacter sp. 8,7 % және Enterococcus faecium 1,2 %. MRSA-да 13,7 %-дан 41,9 %-ға (p=0,041) төзімділіктің айтарлықтай артуы анықталды, K.pneumoniae-де карбапенемдерге төзімділік 0 %-дан 8,3 %-ға (p=0,057) артты, бұл ретте жиіліктің төмендеуін атап өтеміз. K.pneumoniae ESBL түзетін штамдарының таралуы 63,3 %-дан 45,2 %-ға (p=0,058), P.aeruginosa штамдарында карбапенемдерге төзімділік 64,3 %-дан 37,7 %-ға (p=0,037), A.baumannii 48,5 %-дан 19,1 %-ға (p=0,039) төмендеді. Алынған нәтижелер бойынша біздің балалар кардиохирургиясы бөлімінде ESKAPE қоздырғыштары

64,2 % құрады. Ең көп таралған изоляттар S.aureus, K.pneumoniae және A.baumannii болды, K.pneumoniae-нің карбапенемдерге және MRSA-ға төзімділігінің күрт өсу тенденциясы байқалды. Біздің нәтижелеріміз әрбір ауруханада жақсы жобаланған инфекциялық бақылау стратегиясы қажет екенін көрсетті, соның ішінде жақсы гигиеналық стратегия, микробиологиялық мониторинг және ауруханаішілік бақылау.

Кілт сөздер: микробиологиялық мониторинг, ESKAPE микроорганизмдері, таралу, антибиотиктерге төзімділік, балалар кардиохирургиясы.

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Исследование распространенности ESKAPE патогенов и их резистентность к антимикробным препаратам

Проведено проспективное микробиологическое исследование распространенности микроорганизмов ESKAPE и показателей антибиотикорезистентности штаммов у пациентов детского кардиохирургического отделения высокоспециализированной клиники за период с 2019 по 2023 год. В исследовании использовались классические рутинные бактериальные методы выявления изолятов, а также автоматизированную систему окончательной идентификации и тестирования чувствительности Vitek 2 — Compact. В исследование было включено 3725 клинических образцов, частота обнаружения патогенов составила: *S.aureus* – 35,2 %, *K.pneumoniae* – 27,3 %, *A.baumannii* – 14,5 %, *Ps.aeruginosa* – 12,4 %, *Enterobacter sp*. – 8,7 % и *Enterococcus faecium* – 1,2 %. Значительное увеличение резистентности было выявлено у MRSA – с 13,7 % до 41,9 % (p=0,041), у *K.pneumoniae* устойчивость к карбапенемам увеличилась с 0 % до 8,3 % (p=0,057) при этом отмечается снижение частоты распространенности ESBL-продуцирующих штаммов *K.pneumoniae* с 63,3 %. до 45,2 % (p=0,058). Резистентность к карбапенемам у штаммов *P.aeruginosa* снизилась с 64,3 % до 37,7 % (p=0,037), а у *A.baumannii* – с 48,5 % до 19,1 % (p=0,039). По результатам исследования микроорганизмы ESKAPE составили 64,2 %. Наиболее частыми изолятами были *S.aureus, K.pneumoniae* и *A.baumannii*, при этом отмечается резко возрастающая тенденция к устойчивости K.pneumoniae к карбапенемам и MRSA. Полученные результаты подчеркивают необходимость инфекционного контроля в каждой больнице, включающего хорошую стратегию гигиены, микробиологический мониторинг, а также внутрибольничный контроль.

*Ключевые слова***:** микробиологический мониторинг, ESKAPE-микроорганизмы, распространенность, антибиотикорезистентность, детская кардиохирургия.

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