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Dynamics of *Acinetobacter baumannii* isolation and antibiotic resistance in a multidisciplinary clinic for 2021-2025

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Abstract. *Acinetobacter baumannii* is one of the most influential pathogens causing nosocomial infections characterized by high resistance to antibacterial drugs. Thus, this study aimed to evaluate the dynamics of *A. baumannii* isolation and the level of antibiotic resistance in a multidisciplinary hospital for the period 2021-2025. For this reason, a comparative retrospective analysis was conducted on 13 221 clinical isolates obtained from various departments. The analysis was conducted using phenotypic identification and susceptibility testing methods. Susceptibility testing (antibiograma) was performed using the Vitek 2 automated system and the disk diffusion method for nine antimicrobial drugs. These antibiotics included carbapenems (imipenem, meropenem), aminoglycosides (gentamicin, amikacin, tobramycin), fluoroquinolones (ciprofloxacin, levofloxacin), colistin, and a trimethoprim/sulfamethoxazole combination. The results showed a decrease in the frequency of *A. baumannii* isolation over the observation period ($\chi^2 = 19.29$, $p = 0.0007$), with the majority of isolates retaining multiple-drug resistance (MDR). The highest resistance was detected to fluoroquinolones and carbapenems, while susceptibility to colistin remained high throughout all years. The lack of molecular analysis limits the interpretation of the results; however, the resulting resistance profile suggested genes that could act as pathogenicity factors. These comparative retrospective analyses of resistance highlight the need for ongoing local monitoring of A. baumannii antibiotic resistance and the rational use of antimicrobials in hospital settings.

Keywords: *Acinetobacter baumannii,* antibiotic resistance, multidrug resistance (MDR), carbapenems, fluoroquinolones, colistin, clinical isolates

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Introduction

Antibiotic resistance (AR) remains one of the most significant public health threats in the 21st century [1,2]. The widespread spread of resistant pathogens and the declining effectiveness of essential antimicrobials lead to increased mortality, prolonged hospitalization, and increased healthcare costs [1,2]. Among hospital-acquired pathogens, *Acinetobacter baumannii* poses a particular danger [3–6]. It is a non-fermenting Gram-negative rod characterized by its ability to survive in the hospital environment [3–6]. Its rapidly accumulating resistant determinants cause severe nosocomial infections (including ventilator-associated pneumonia and bacteremia) with high mortality in intensive care units [3–6].

Current systematic reviews and meta-analyses demonstrate wide geographic variability in *A. baumannii* resistance levels [3,4]. At the same time, many regions exhibit persistently high rates of resistance to carbapenems, fluoroquinolones, and aminoglycosides. Carbapenem-resistant *A. baumannii* (CRAB) strains are listed by the World Health Organization (WHO) as priority bacterial pathogens requiring the development of new therapeutic agents and enhanced surveillance [7,8]. Furthermore, there are alarming reports of an increase in cases resistant even to reserve drugs (colistin, tigecycline) [9–14]. Molecular epidemiological analyses highlight the role of clinical clones and plasmid-mediated transmission of resistance genes in the formation of local and interregional outbreaks [11,15,16].

Reliable epidemiological surveillance of AR requires continuous monitoring through the analysis of obtained data, which allows us to separate random annual fluctuations from stable trends [17–20]. Timely detection of outbreaks of clonal spread and assessment of the impact of interventions (antibiotic stewardship, infection control measures) are important for sustainable control [17–20]. Thus, local and regional studies assessing the annual dynamics of S/R for individual antibiotics and the distribution of non-MDR (non-multi-drug-resistant)/ MDR (multi-drug-resistant)/PDR (pan-drug-resistant) categories are a valuable source of clinical and epidemiological information [1,8,12]. They inform empirical treatment regimens, antimicrobial stewardship policies, and infection control measures in hospitals [1,8,12]. With limited therapeutic options and the slow development of new antibiotics, timely monitoring and transparent reporting remain crucial elements in responding to the A. baumannii threat.

Materials and research methods

Object and research basis

The study involved clinical isolates of *Acinetobacter baumannii* isolated from various biological specimens of patients hospitalized at the National Research Medical Center (NRMC) between 2021 and 2025. The study included specimens from the anesthesiology and intensive care departments (ICU), urology, surgery, and therapy patients. In some cases, specimens were received from the microbiology department (MOB-2). Over five years, a total of 13 221 clinical specimens were analyzed, from which a varying number of A. baumannii isolates were identified by year (Table 1).

Table 1

Annual distribution of the number of tested samples and positive isolates of *A. baumannii* at the NRMC (2021-2025)

Year	Number of samples tested (n)	*A. baumannii* isolates (n)	Main departments where the pathogen was isolated
2021	2 939	12	ICU, urology, outpatient department, day hospital

2022	2 746	28	ICU, urology, surgery, and the outpatient department
2023	2 505	16	Urology, internal medicine, outpatient clinic, surgery
2024	2 739	12	Urology, surgery, outpatient clinic, ICU
2025	2 292	6	ICU, Urology, CCD, Urology, MOB-2

Note: the calculation was based on data from the microbiological registry of the NRMC (2021-2025). ICU – intensive care departments, MOB-2 – microbiology department.

Selection of biological materials

Between 2021 and 2025, the study included clinical specimens collected from patients in various departments of the NRMC (Table 1). The predominant samples examined were from the urinary tract (urine), the respiratory tract (bronchial washings, sputum, throat swabs, pleural fluid, and contents of intubation and suction catheters), as well as wound, drainage, wound and pressure ulcer swabs, and contents of central venous catheters. All specimens were collected using an aseptic technique and transported to the microbiology laboratory within two hours after collection [21].

Microbiological analysis and identification of isolates

Clinical specimens were cultured on standard nutrient media, including blood agar, Endo agar, egg yolk-salt agar, Kalina agar, chocolate agar, and chromogenic media. Incubation was performed at 37°C for 18-24 hours. Preliminary identification of microorganisms was performed by morphological characteristics, Gram staining, and biochemical tests [21]. Pure cultures were also identified using the automated Vitek 2 Compact system [22,23].

Antimicrobial susceptibility testing (antibiograma) of *A. baumannii* isolates was performed using two methods. The first method was a disk diffusion assay on a nutrient medium using commercial antibiotic disks [22]. Inhibition zones were recorded after 18-24 hours of incubation at 37°C and interpreted according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [24]. The second method was automated using the Vitek 2 Compact system [23].

The comparative retrospective analyses of resistance included drugs from various pharmacological groups: imipenem, meropenem, amikacin, gentamicin, tobramycin, ciprofloxacin, levofloxacin, trimethoprim/sulfamethoxazole, and colistin. Results were interpreted according to EUCAST criteria, classifying isolates as intermediate (S), intermediate (I), or resistant (R) [24]. Intermediate (I) and resistant (R) isolates were combined during the analysis to compare intermediate and resistant isolates.

Statistical data analysis

Statistical data processing was performed using GraphPad Prism 8.0.1. For each year, the proportion of positive A. baumannii isolates among the total number of tested samples was calculated, expressed as a percentage. Based on these data, the mean proportion (%) and standard error of the mean (SE) were determined and used to plot error bars (values are presented as mean ± SE). Comparisons of proportions between groups were performed using the χ^2 (chi-square) test or Fisher's exact test, depending on the sample size [21]. Differences were considered statistically significant at $p < 0.05$. All graphical representations included legends indicating the analysis method used and confidence levels.

Results

The proportion of A. baumannii among clinical isolates in 2021-2025

The analysis revealed a significant downward trend in the frequency of *Acinetobacter baumannii* isolation from patient clinical specimens over the period 2021-2025. The proportion of positive isolates was 1.09% in 2021, 1.02% in 2022, 0.64% in 2023, 0.44% in 2024, and 0.26% in 2025, representing a total of 4.49% across the five years. The highest number of *A. baumannii* cases was recorded in 2021-2022. The decrease in the proportion of the pathogen in subsequent years likely reflects the effectiveness of infection control measures and the rationalization of antibacterial therapy in the hospital. The statistical analysis performed using the χ^2 method confirmed the reliability of differences between the years of observation (χ^2 = 19.29; df = 4; p = 0.0007). This indicates a statistically significant decrease in the proportion of *A. baumannii* isolates over the five-year observation period.

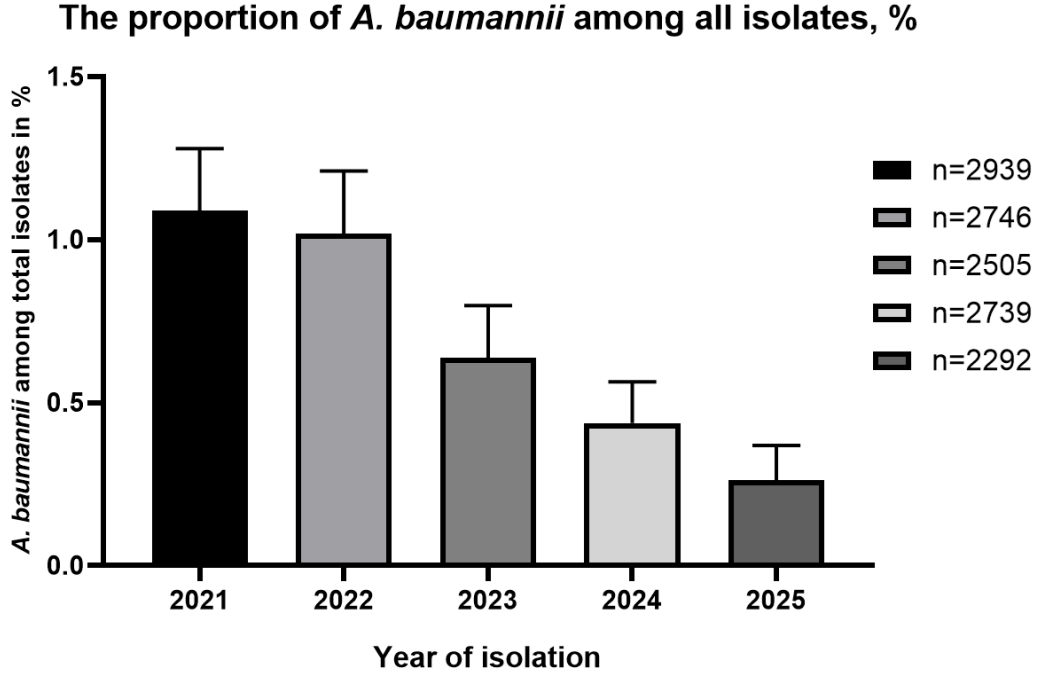


Figure 1. Yearly changes in the proportion of *Acinetobacter baumannii* among all isolates (2021-2025). The total number of isolates (n) for each year is indicated in the diagram. The diagram shows values ± standard error of proportion (SE). Statistical analysis was performed using the chi-square (χ^2) test comparing positive and negative *A. baumannii* cases across the years 2021-2025 (χ^2 = 19.29; df = 4; p = 0.0007)

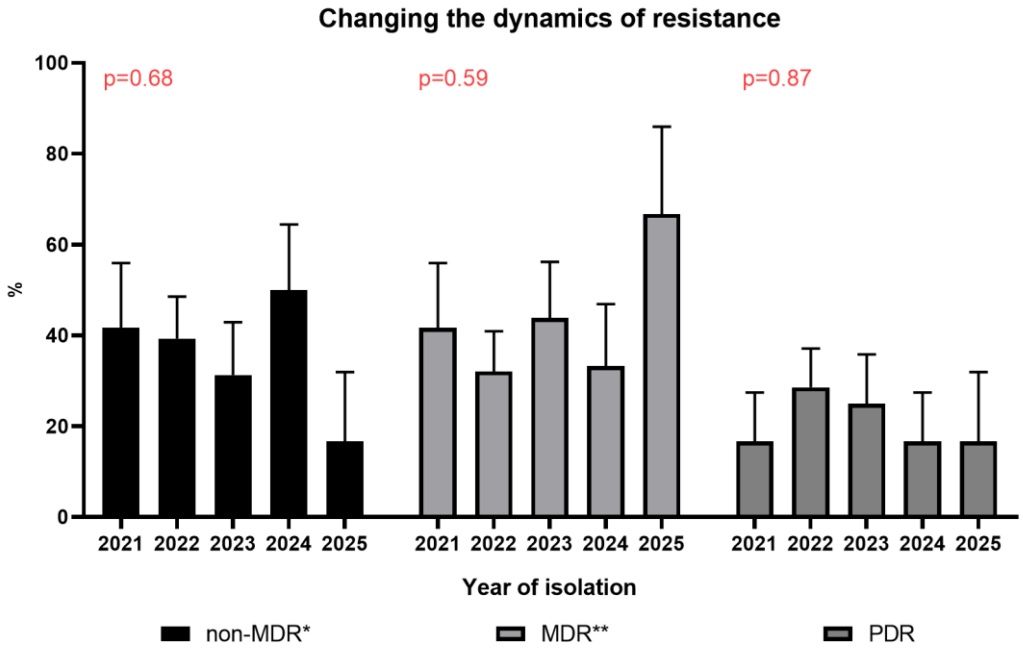
Changes in the resistance structure of A. baumannii isolates

An analysis of the distribution of *A. baumannii* isolates by resistance categories (non-MDR*, MDR**, and PDR) showed that multidrug-resistant strains predominated in all observation years (Figure 2). In 2021, the proportion of MDR isolates was approximately 42%, non-MDR 25%, and PDR 8%. In subsequent years, slight fluctuations between categories were observed, but without significant dynamics (p > 0.05). Overall, the resistance pattern remained stable throughout the study period. At the same time, the proportion of MDR isolates remained high, indicating the persistence of resistant clones of the pathogen in hospital settings. The recorded

periodic variability in the ratios of non-MDR and PDR strains likely reflects the influence of factors associated with changes in antibiotic therapy tactics, drug rotation, and hospital environmental characteristics. Despite the absence of an increase in PDR forms, the persistence of a significant proportion of MDR isolates poses a serious problem for clinical practice.

Antibiotic susceptibility of A. baumannii isolates

The results of antibiotic susceptibility testing of *A. baumannii* isolates collected between 2021 and 2025 indicate widespread drug resistance and a strong tendency toward the persistence of a multidrug-resistant phenotype. The overall picture (Figure 3) shows that *A. baumannii* exhibited low susceptibility to most antimicrobial agents. The exception was colistin, whose activity remained high over the last three years of observation. However, the data were not presented graphically due to the small sample size.



Note: *Because there were only 3 cases of *A. baumannii* that were completely susceptible to antibiotics during the entire observation period, it was combined into a group as non-MDR, which includes those resistant to 1 and 2 antibiotics. **The MDR group included resistance to equal or more than 3 antibiotics. The PDR group includes isolates that were resistant to all antibiotics tested.

Figure 2. Yearly changes in the resistance dynamics of *Acinetobacter baumannii* among all isolates (2021, n=12; 2022, n=28; 2023, n=16; 2024, n=12; 2025, n=6). The diagram shows values ± standard error of proportion (SE). Statistical analysis was performed using the chi-square (χ^2) test comparing response type with other *A. baumannii* cases across the years 2021-2025

Aminoglycosides

Susceptibility of isolates to amikacin was 58% in 2021, decreased to 50% in 2022, and reached a minimum of 33% by 2025. A similar trend was observed for gentamicin. In 2021, the proportion of susceptible strains was 58%, in 2023 it was 50%, and by 2025 it had decreased to 20%. Similar fluctuations were observed for tobramycin, from 70% of isolates susceptible in 2021 to 30% in 2025. Although the differences between years did not reach statistical significance ($p > 0.05$), the overall trend indicates a gradual decline in aminoglycoside activity.

This indicates the emergence of resistance in the hospital strain population. The high level of cross-resistance between amikacin, gentamicin, and tobramycin indicates the accumulation of similar genetic determinants (aacC, aphA, and aadB) typical for *A. baumannii* [25,26].

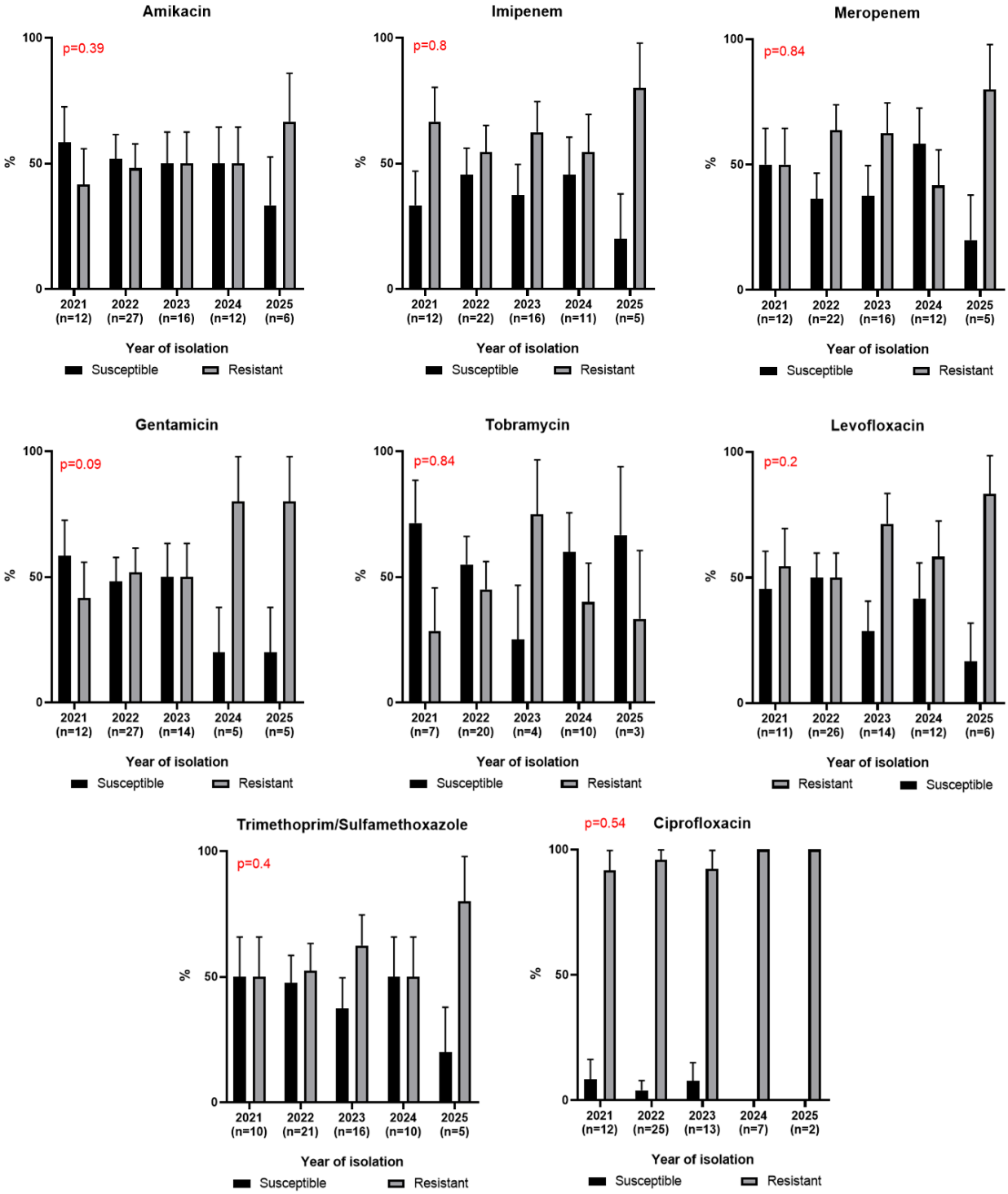


Figure 3. Changes in the sensitivity of *A. baumannii* isolates to antibiotics from various pharmacological groups from 2021 to 2025. The diagram shows values ± standard error of proportion (SE). Statistical analysis was performed using the chi-square (χ^2) test to compare susceptible and resistant *A. baumannii* cases across the years 2021-2025

Carbapenems

The most alarming situation is observed for imipenem and meropenem. In 2021, susceptibility to imipenem was 33%, by 2024 it was 45%, and by 2025 it had decreased to 20%. Similar trends were observed for meropenem (50%, 58%, and 20%, respectively). These data confirm the presence of high levels of carbapenem resistance, likely associated with the spread of OXA-type D carbapenemases (primarily OXA-23-like, OXA-24/40-like и OXA-58-like), consistent with global trends [7,27–29]. Carbapenem-resistant strains typically exhibit multiple resistance and limited therapeutic options, as evidenced by the high correlation with the MDR and PDR categories in this study.

Fluoroquinolones

Fluoroquinolone activity remained low throughout the study period. While susceptibility to levofloxacin was 45% in 2021, 30% in 2023, and decreased to 17% by 2025, susceptibility to ciprofloxacin did not exceed 10% in all observation years. These values indicate the practical ineffectiveness of fluoroquinolones against clinical isolates of *A. baumannii*. This is likely due to mutations in the gyrA and parC genes, as well as activation of the efflux systems [30,31].

Sulfonamides

For trimethoprim/sulfamethoxazole, moderate fluctuations in susceptibility were observed, ranging from 50% (2021) to 20% (2025). Although there were no statistically significant differences ($p > 0.05$), a gradual decrease in susceptibility persisted. Given the limited use of this drug in hospital settings, the decrease in activity may be due to cross-resistance via plasmid determinants such as dfrA1, dfrA5, sul1, and sul2 [26,29].

Polymyxins

Of particular importance is the high activity of colistin, to which over 60% of isolates remained susceptible throughout the observation period (2023-2025). In 2024-2025, susceptibility increased to 80% and 100%, respectively. This makes colistin a "last resort" drug for the treatment of infections caused by MDR/XDR *A. baumannii* strains. However, the development of resistance to polymyxins remains extremely rare. This is likely due to the limited use of the drug and the high fitness cost of mutations in the lpxA/D, lpxACD, and pmrB genes [32,33].

Discussion

The results of this study demonstrate that, over the period 2021-2025, a clear downward trend in the frequency of *A. baumannii* isolation among all clinical isolates was observed in a large multidisciplinary hospital. The significant decrease in the proportion of positive isolates ($\chi^2 = 19.29$; $p = 0.0007$) may reflect the effectiveness of the implemented infection control measures and optimization of antibacterial therapy in various departments. Similar trends have been described in a number of publications where the implementation of antimicrobial stewardship programs led to a reduction in the prevalence of hospital isolates of *A. baumannii* [3,5,12].

Despite the positive trend in the reduction of pathogen isolation frequency, the structure of drug resistance in *A. baumannii* remained tense. MDR strains predominated in all study years, which is consistent with the results of similar observations conducted in other countries [2,6,10]. The high proportion of MDR strains indicates stable circulation of hospital clones possessing a combination of defense mechanisms [4,11]. This is possibly associated with the rapid production of broad-spectrum β-lactamases, the activity of efflux systems, and changes in the permeability of the outer membrane [4,11].

The most pronounced resistance was observed to carbapenems (imipenem, meropenem) and fluoroquinolones, which is consistent with international observations, in which the spread of carbapenem-resistant clones of A. baumannii (CRAB) is considered one of the main challenges of clinical microbiology [7,14,32]. Given the lack of molecular typing in our study, it is assumed that the obtained phenotypic manifestations are associated with the spread of OXA-type β-lactamases [7,27–29]. We also assume that this is associated with possible mutations in the gyrA and parC genes and the activation of adeABC-type efflux systems, which has been confirmed by other authors [34–36].

An interesting feature is the persistence of high susceptibility of isolates to colistin, which is consistent with the results of studies conducted in Europe and the Middle East [6,37,38]. The lack of growth of colistin-resistant strains in our study is likely due to the limited use of this drug in clinical practice. Mutations in the pmrA/pmrB and lpxACD systems responsible for resistance to polymyxins are known to be accompanied by a significant fitness cost, which reduces the likelihood of their persistence in the population [32,33].

It should be emphasized that the study was phenotypic in nature and did not include molecular analysis of resistance genes. This limits the ability to accurately identify resistance mechanisms and genetically characterize circulating clones. However, the results obtained allow us to formulate hypotheses about the likely mechanisms of multiple resistance. This, in turn, allows us to formulate a direction for further research, including genome sequencing and the identification of pathogenicity factors (OXA, NDM, sul, aad, adeABC, etc.). From a practical standpoint, the data confirm the need for continuous local monitoring of *A. baumannii* susceptibility. Notably, phenotypic shifts can serve as an early indicator of the emergence of new resistant clones. Going forward, it seems advisable to combine epidemiological observations with molecular genetic methods (WGS, MLST) to clarify clonal affiliation and sources of nosocomial spread. In the future, the results of this study can be used to adjust empirical treatment regimens, optimize antimicrobial control programs, and develop regional protocols for the rational use of antibiotics.

Conclusion

The study assessed the isolation dynamics and antibiotic resistance levels of *A. baumannii* in hospitals from 2021 to 2025. The comparative retrospective analyses of resistance data obtained indicate a decrease in the rate of pathogen isolation. This may reflect the effectiveness of implemented infection control measures and the rationalization of antibacterial therapy. However, despite this positive trend, a high level of multidrug resistance (MDR) persists, including to β-lactam antibiotics and carbapenems, confirming the persistent circulation of hospital clones. Of particular note is the high susceptibility of isolates to colistin, which persisted throughout the observation period. This is likely due to the limited use of the drug and the high fitness cost of mutations conferring resistance to polymyxins. Meanwhile, moderate susceptibility rates to trimethoprim/sulfamethoxazole may be due to cross-resistance associated with plasmid determinants of the sul1/sul2 type. It should be noted that this study was phenotypic in nature and did not include molecular verification of resistance mechanisms. This limits the ability to accurately identify resistance determinants and assess the clonal structure of the population. Nevertheless, the identified patterns allow us to hypothesize possible mechanisms of multiple resistance and outline areas for further research, including molecular genetic methods to detect pathogenicity factors. The results of this study can potentially be used to improve surveillance systems for hospital-acquired infections, optimize empirical antibiotic therapy, and develop

regional antimicrobial control protocols. Continued studies using molecular technologies will provide a deeper understanding of the spread of resistance and develop more effective strategies for its containment.

Author Contributions

N.S.S., N.B., and **M.D. –** conceptualization and design of the study; **N.S.S.** and **N.B.** –methodology and visualization; **N.S.S., N.B., M.D.,** and **A.B.** – writing – original draft; **N.S.S., N.B., M.A., S.A., A.B.,** and **G.D. –** writing – review & editing; **N.S.S., G.D., M.A., A.S.** – investigation, data curation, and formal analysis.

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Conflicts of Interest

The authors declare no conflicts of interest.

Compliance with ethical standards

All procedures performed in studies involving people comply with the ethical standards of the National Committee on Research Ethics and the 1964 Helsinki Declaration and its subsequent amendments or comparable ethical standards.

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Көпбейінді ауруханадағы 2021-2025 жылдар аралығында *Acinetobacter baumannii* оқшаулану жиілігі және антибиотикке төзімділігінің динамикасы

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Аңдатпа. *Acinetobacter baumannii* – ауруханаішілік инфекцияларды тудыратын ең қауіпті патогендердің бірі, ол бактерияға қарсы препараттарға жоғары деңгейдегі төзімділігімен сипатталады. Сондықтан, бұл зерттеудің мақсаты 2021-2025 жылдар аралығындағы көп салалы ауруханада *A. baumannii* оқшаулану динамикасын және антибиотикке төзімділік деңгейін бағалау болды. Осы мақсатта әртүрлі бөлімдерден алынған 13 221 клиникалық изоляттың салыстырмалы ретроспективті талдауы жүргізілді. Талдау фенотиптік идентификация және антибиотиктерге сезімталдықты тексеру арқылы жүргізілді. Сезімталдықты тексеру (антибиограммалар) автоматтандырылған Vitek 2 жүйесі және тоғыз микробқа қарсы агент үшін диск диффузиясы әдісін қолдану арқылы жүргізілді. Бұл антибиотиктерге карбапенемдер (имипенем, меропенем), аминогликозидтер (гентамицин, амикацин, тобрамицин), фторхинолондар (ципрофлоксацин, левофлоксацин), колистин және триметоприм/сульфаметоксазол комбинациясы жатады. Нәтижелер саралау кезеңінде *A. baumannii* оқшаулану жиілігінің төмендегенін көрсетті ($\chi^2 = 19.29$, $p = 0.0007$), көптеген изоляттар көп дәрілік төзімділікті (КДР) сақтап қалды. Фторхинолондар мен карбапенемдерге ең жоғары төзімділік анықталды, ал колистинге сезімталдық барлық жылдар бойы жоғары болып қалды. Молекулалық талдаудың болмауы нәтижелерді түсіндіруді шектейді. Дегенмен, алынған төзімділік профилі патогенділік факторлары ретінде әрекет етуі мүмкін гендердің бар екенін көрсетеді. Салыстырмалы ретроспективті төзімділік талдауының нәтижелері *A. baumannii* антибиотиктерге төзімділігін үздіксіз жергілікті бақылау және аурухана жағдайында микробқа қарсы препараттарды ұтымды пайдалану қажеттілігін көрсетеді.

Түйін сөздер: *Acinetobacter baumannii,* антибиотиктерге төзімділік, көп дәрілік төзімділік (КДР), карбапенемдер, фторхинолондар, колистин, клиникалық изоляттар

Динамика выделения *Acinetobacter baumannii* и антибиотикорезистентности в многопрофильном стационаре за 2021-2025

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Аннотация. *Acinetobacter baumannii* является одним из наиболее опасных возбудителей нозокомиальных инфекций, характеризующихся высокой устойчивостью к антибактериальным препаратам. В связи с этим целью данного исследования была оценка динамики выделения *A. baumannii* и уровня антибиотикорезистентности в многопрофильном стационаре за период 2021-2025 гг. Для это был проведен сравнительный ретроспективный анализ 13 221 клинических изолятов, полученных из различных отделений. Анализ проводился с использованием методов фенотипической идентификации и определения чувствительности к антибиотикам. Определение чувствительности (антибиограммы) проводилось с помощью автоматизированной системы Vitek 2 и диско-диффузионного метода для девяти антимикробных препаратов. К этим антибиотикам относятся карбапенемы (имипенем, меропенем), аминогликозиды (гентамицин, амикацин, тобрамицин), фторхинолоны (ципрофлоксацин, левофлоксацин), колистин и комбинация триметоприма/сульфаметоксазола. Результаты показали снижение частоты выделения A. baumannii за период наблюдения (χ^2 = 19.29, p = 0.0007), при этом большинство изолятов сохраняли множественную лекарственную устойчивость (МЛУ). Наибольшая резистентность была выявлена к фторхинолонам и карбапенемам, в то время как чувствительность к колистину оставалась высокой на протяжении всех лет. Отсутствие молекулярного анализа ограничивает интерпретацию результатов, однако полученный профиль резистентности позволяет предположить наличие генов, которые могут выступать в качестве факторов патогенности. Результаты сравнительного ретроспективного анализа резистентности подчеркивают необходимость постоянного локального мониторинга резистентности *A. baumannii* к антибиотикам и рационального использования антимикробных препаратов в условиях стационара.

Ключевые слова: *Acinetobacter baumannii,* устойчивость к антибиотикам, множественная лекарственная устойчивость (МЛУ), карбапенемы, фторхинолоны, колистин, клинические изоляты

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