Trends of Colistin MIC among Acinetobacter baumannii and Pseudomonas aeruginosa at a first-class hospital in Vietnam

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Trends of Colistin MIC among *Acinetobacter baumannii* and *Pseudomonas aeruginosa* at a first-class hospital in Vietnam

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Abstract

Introduction

A. baumannii and *P. aeruginosa* belong to the multidrug-resistant Gram-negative bacteria group, posing significant challenges in treatment. Colistin is considered the last-line antibiotic for treating this bacterium. It is essential to determine the minimum inhibitory concentration (MIC) to adjust the appropriate dosage.

Method

A cross-sectional descriptive study using data (January 2020 – August 2022) was conducted.

Results

A. baumannii and *P. aeruginosa*, accounting for 15.5% and 9.4% of common multidrug-resistant Gram-negative bacteria, respectively, showed an increasing trend over the years. *A. baumannii* exhibited higher resistance rates than *P. aeruginosa* with tested antibiotics. Both bacterial species remained completely sensitive (100%) to Colistin. In the Colistin MIC values within the sensitive range, *A. baumannii* and *P. aeruginosa* showed a trend of increasing MIC values over the years ($p<0.001$). Notably, *P. aeruginosa* showed a more pronounced change than *A. baumannii*, with a higher percentage of strains having MIC values of 1 μg/mL and 1.5 μg/mL. ($p<0.001$). There was no difference in Colistin MIC between carbapenem-resistant and non-resistant with *A. baumannii* (Imipenem and Meropenem). *P. aeruginosa* resistant to Imipenem tended to have higher MIC values than non-resistant strains ($p<0.001$).

Conclusions

A. baumannii and *P. aeruginosa* showed an upward trend of Colistin MIC values over the years, indicating these bacterial strains are gradually becoming resistant to Colistin over time. It is essential to implement control measures of Colistin-resistant strains in the future.

Key words: Colistin MIC; *A.baumannii*; *P.aeruginosa*

Introduction

Antibiotic resistance is currently one of the most globally concerning health issues. Jim O'Neill's comprehensive report on the worldwide antibiotic resistance situation estimates that by 2050, there will be over 10 million deaths globally due to drug-resistant bacteria, resulting in damages exceeding 100 trillion dollars in treatment costs. In this context, Asia is considered the region with the highest rates of antibiotic-resistant bacteria, especially in developing countries like Vietnam [1]. Reports on the antibiotic resistance situation in Vietnam have documented the widespread prevalence of Gram-negative bacteria in the majority of healthcare facilities, showing high resistance to commonly used antibiotics [2].

Among the common Gram-negative bacteria, the most prominent are *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Both are listed by the World Health Organization (WHO) as multidrug-resistant bacteria requiring top priority in research and development of new antibiotic drugs. These bacteria are known to be among the superbugs causing multidrug-resistant diseases, exhibiting broad resistance to various commonly used antibiotics such as β-Lactams, Aminoglycosides, and Fluoroquinolones. They also possess a high survival capability under various environmental conditions [3,4]. These factors have posed considerable challenges in the treatment of infections caused by *A. baumannii* and *P. aeruginosa*. In this situation, Colistin is considered one of the last-line antibiotics for treating multidrug-resistant Gram-negative strains [5]. Besides its pharmacological effects, Colistin also exhibits certain side effects on the human body, particularly nephrotoxicity [6]. Consequently, administering Colistin to patients requires maintaining drug concentrations through the assessment of minimum inhibitory concentration (MIC) to adjust appropriate doses for effective bacterial eradication while minimizing drug toxicity. In recent years, cases of Gram-negative bacillus being clinically susceptible to Colistin have been documented in the medical literature [7,8]. Based on many complex bacterial antibiotic resistance mechanisms, plus inappropriate antibiotic use in some places, Colistin's MIC levels have shifted in an upward direction over time. Mild to severe colistin resistance has been reported to be associated with expression of mcr-family

genes, especially mcr-1 [9]. Therefore, in addition to helping to limit the toxicity of the drug, periodic monitoring of the MIC of this antibiotic in healthcare facilities also helps to monitor the trend and detect Colistin resistance early.

Currently, reports on the antibiotic resistance situation in Vietnam primarily focus on the sensitivity and resistance rates of *A. baumannii* and *P. aeruginosa*, with limited attention given to the variations in Colistin MIC values over the years. This emphasizes the need for research into the changes in Colistin MIC values for these two bacterial species. Such research is crucial for implementing effective antibiotic management strategies to prevent the escalation of resistance. This study aims to determine the trends in common MIC values of Colistin for *A. baumannii* and *P. aeruginosa* in clinical specimens from 2020 to 2022.

Methodology

Ethical Approval

The research was accepted by the ethical principles established by the Ethics Committee for Biomedical Research at Nguyen Tri Phuong Hospital, as indicated by document number 746/NTP-HĐĐĐ, dated April 25, 2023.

Study design

This cross-sectional descriptive study used recorded data from January 2020 to August 2022

Bacterial Strains

Data on antibiotic sensitivity for 1073 strains of *A. baumannii* and 653 strains of *P. aeruginosa* were isolated from various clinical samples at Nguyen Tri Phuong Hospital, Ho Chi Minh City, Vietnam, from January 2020 to August 2022. The bacterial strains were identified and subjected to antibiotic susceptibility testing following the standard microbiological procedures of the hospital's Laboratory Department. All samples provide comprehensive information including clinical department, cultured specimen type, bacterial identification results, and antibiotic susceptibility profile. Additionally, Minimum Inhibitory Concentration (MIC) testing with Colistin was performed.

Bacterial Cultivation and Isolation

The clinical specimens were cultured on suitable nutrient media, including Blood Agar (BA), Chocolate Agar (CA), and Mac Conkey Agar (MC), using various cultivation techniques. For sputum and urine specimens, quantitative cultures were conducted on nutrient media, and the bacterial colonies were isolated for analysis when the colony count exceeded 10^4 CFU/ml. For other specimen types, both isolation and analysis of all bacterial colonies were performed.

Bacterial Identification

The selected bacterial colonies underwent Gram staining. Based on the Gram staining results and the type of growth medium, Gram-negative bacterial colonies were selected for identification using the IDS GN15 kit (Nam Khoa Biotek, Vietnam). Bacterial samples were incubated at 37°C for 18-20 hours, and the results of the tested biochemical reactions were used to determine the scientific names of the bacteria.

Antibiotic Susceptibility Testing

To determine bacterial sensitivity to antibiotics, the disk diffusion method on Mueller-Hinton agar (MHA) was employed (Kirby-Bauer technique). The selected bacterial colonies were diluted with physiological saline to create an inoculum with 0.5 McFarland. The suspension was spread onto MHA plates, and antibiotic disks (Nam Khoa Biotek, Vietnam) were placed on the agar. Antibiotic disks were selected based on clinical needs and the suspected bacterial species as follows:

For bacteria suspected to belong to the *Pseudomonas spp* genus (with positive Oxidase reaction): Place antibiotic disks including Piperacillin/tazobactam, Amoxicillin/clavulanate, Ticarcillin/clavulanate, Trimethoprim/sulfamethoxazole, Amikacin, Gentamicin, Tobramycin, Netilmicin, Ciprofloxacin, Levofloxacin, Ceftazidime, Cefepime, Imipenem, Meropenem, Colistin.

For bacteria suspected to belong to the *Acinetobacter spp* genus (with negative Oxidase reaction): Place antibiotic disks including Piperacillin/tazobactam, Ampicillin/sulbactam, Trimethoprim/ sulfamethoxazole,

Doxycycline, Gentamicin, Tobramycin, Ciprofloxacin, Levofloxacin, Cefotaxime, Ceftazidime, Cefepime, Imipenem, Meropenem, Colistin.

Incubate the Mueller-Hinton agar (MHA) plates at 37°C for a duration of 18 to 20 hours. Compare the measured zone diameters with the interpretive criteria outlined in the Clinical and Laboratory Standards Institute (CLSI) 2019 guidelines [10].

Minimum Inhibitory Concentration of Colistin (Colistin MIC)

The minimum inhibitory concentration (MIC) of Colistin was determined using the E-Test method (Epsilometer test Epsilon) with reagents supplied by Nam Khoa Biotek, Vietnam. This method operates on the principle of using strips with a gradually decreasing antibiotic concentration along their length. When these strips were placed on an evenly inoculated agar plate with bacteria, the bacteria grew until the end of the strip with a low antibiotic concentration, but they did not grow at the high antibiotic concentration end of the strip. The point where the first bacterial growth touches the strip was used to calculate the MIC. The strip had a numerical scale of concentrations directly marked on it to assist in determining the MIC. The Mueller-Hinton agar (MHA) plates were incubated at 37°C for 18-20 hours, and the MIC values of Colistin were read. The results were interpreted following the guidelines provided by CLSI 2019 [10].

Statistical Analysis

The data was analyzed by STATA 14.2. The analysis results were presented in tables and charts depicting the positive rates by bacterial species, positive rates by specimen type, the distribution of Colistin MIC values (MIC $\leq$0.75 μg/mL, MIC =1.0 μg/mL, MIC = 1.5 μg/mL, and MIC =2.0 μg/mL) from 2020 to 2022, and the rates of susceptibility (S: susceptible), intermediate (I: intermediate), and resistance (R: resistance) to the tested antibiotics. The Chi-square test was employed to compare differences in the rates of Colistin MIC values and between strains resistant and non-resistant to Carbapenem (Imipenem and Meropenem), with a significance level set at $p < 0.05$ considered statistically significant.

Results

Positive culture rates

A. baumannii and *P. aeruginosa* in the total common multidrug-resistant Gram-negative bacteria accounted for 15.5% and 9.4%, respectively. Those rates increased from 2020 to 2022 (**Table 1**). These bacterial strains were isolated from various clinical specimens, as illustrated in **Figure 1**. The respiratory specimen group had the highest positivity rates for both bacteria, followed by the pus-fluid specimen group, urine specimens, and blood specimens. There was no significant difference in the positivity rates based on the type of clinical specimen between *A. baumannii* and *P. aeruginosa*.

Table 1. Positive culture rates of A. baumannii and P. aeruginosa from 01/2020 to 08/2022

Bacteria	2020		2021		2022		Total of 3 years	
	N	%	N	%	N	%	N	%
A.baumannii	377	13.7%	371	16.0%	325	17.4%	1073	15.5%
P.aeruginosa	242	8.8%	216	9.3%	195	10.5%	653	9.4%
Common multidrug-resistant Gram-negative bacteria (*)	2755	100%	2315	100%	1863	100%	6933	100%

(*) Common multidrug-resistant Gram-negative bacteria consists of A.baumannii, P.aeruginosa, K.pneumoniae, E.coli and Enterobacter spp.

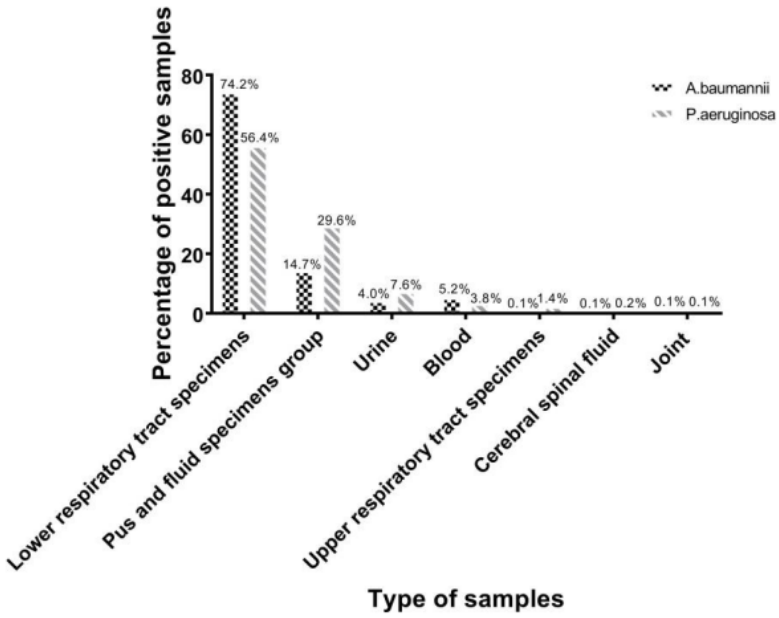

Figure 1. The positive culture rate of *A. baumannii* and *P. aeruginosa* by type of clinical specimen

The lower respiratory tract specimen includes sputum, bronchoalveolar lavage fluid, and endotracheal aspirate. The pus and fluid specimen group includes all types of pus, pleural fluid, peritoneal fluid, pericardial fluid, ascitic fluid, and catheter tip. The upper respiratory tract specimen consists of throat swabs and nasal discharge.

Antibiotic susceptibility

The bacterial strains were tested for antibiotic susceptibility and interpreted according to CLSI 2019 (**Table 2**). *A. baumannii* exhibited a higher resistance rate to the tested antibiotics than *P. aeruginosa*. Specifically, *A. baumannii* showed resistance rates over 90% to representatives of the Fluoroquinolone group (Ciprofloxacin and Levofloxacin) and the β-Lactam group (Cefotaxime, Ceftazidime, Cefepime, Imipenem, Meropenem). *P. aeruginosa* demonstrated resistance rates below 50% to most tested antibiotics except for Amoxicillin/clavulanate and Trimethoprim/sulfamethoxazole. The resistance rate of *A. baumannii* and *P. aeruginosa* to Colistin was 0% from 01/2020 to 08/2022. Therefore, we conducted an

additional survey on the distribution of Colistin MIC values within the susceptible range for these two bacterial species.

Table 2. The resistance rates to the tested antibiotics of A. baumannii and P. aeruginosa.

Tested antibiotics	*A.baumannii*			*P.aeruginosa*		
	S	I	R	S	I	R
Piperacillin/tazobactam	4.9%	2.8%	92.2%	77.9%	13.6%	8.5%
Amoxicillin/clavulanate	Not tested			2.6%	0.0%	97.4%
Ampicillin/sulbactam	16.5%	9.2%	74.3%	Not tested		
Ticarcillin/clavulanate	Not tested			57.0%	18.4%	24.6%
Trimethoprim/sulfamethoxazole	23.3%	6.6%	70.0%	3.5%	6.9%	89.6%
Doxycycline	54.0%	15.0%	31.0%	Not tested		
Amikacin	Not tested			80.3%	0.5%	19.2%
Gentamicin	13.6%	1.7%	84.6%	69.3%	4.3%	26.3%
Tobramycin	13.6%	1.7%	84.6%	75.0%	0.8%	24.2%
Netilmicin	Not tested			78.5%	0.7%	20.8%
Ciprofloxacin	2.8%	1.3%	95.9%	59.8%	6.1%	34.1%
Levofloxacin	4.1%	2.8%	93.0%	54.0%	10.7%	35.3%
Cefotaxime	0.3%	5.3%	94.5%	Not tested		
Ceftazidime	5.0%	1.8%	93.2%	75.2%	1.4%	23.3%
Cefepime	5.8%	1.0%	93.2%	77.3%	0.5%	22.2%
Imipenem	6.6%	0.4%	93.0%	75.3%	2.4%	22.2%
Meropenem	5.7%	0.3%	94.0%	67.3%	3.4%	29.3%
Colistin	100.0%	0.0%	0.0%	100.0%	0.0%	0.0%

Minimum inhibitory concentration (MIC) of Colistin

During the three-year period from 2020 to 2022, the MIC of Colistin was within the sensitive range (Colistin MIC ≤ 2 μg/mL) according to CLSI 2019. The distribution of MIC levels among *A. baumannii* and *P. aeruginosa* strains (**Table 3**) revealed that Colistin MIC values ≤0.75 μg/mL were the most common for both bacterial types. Higher Colistin MIC values corresponded to progressively lower proportions. Notably, *P. aeruginosa* demonstrated a tendency to shift Colistin MIC values closer to 2 μg/mL compared to *A. baumannii*, with a higher concentration of Colistin MIC values at 1 μg/mL and 1.5 μg/mL ($p<0.001$). The changes in the distribution of MIC values of Colistin for *A. baumannii* and *P. aeruginosa* from January 2020 to August 2022 were presented in **Figures 2 and 3**.

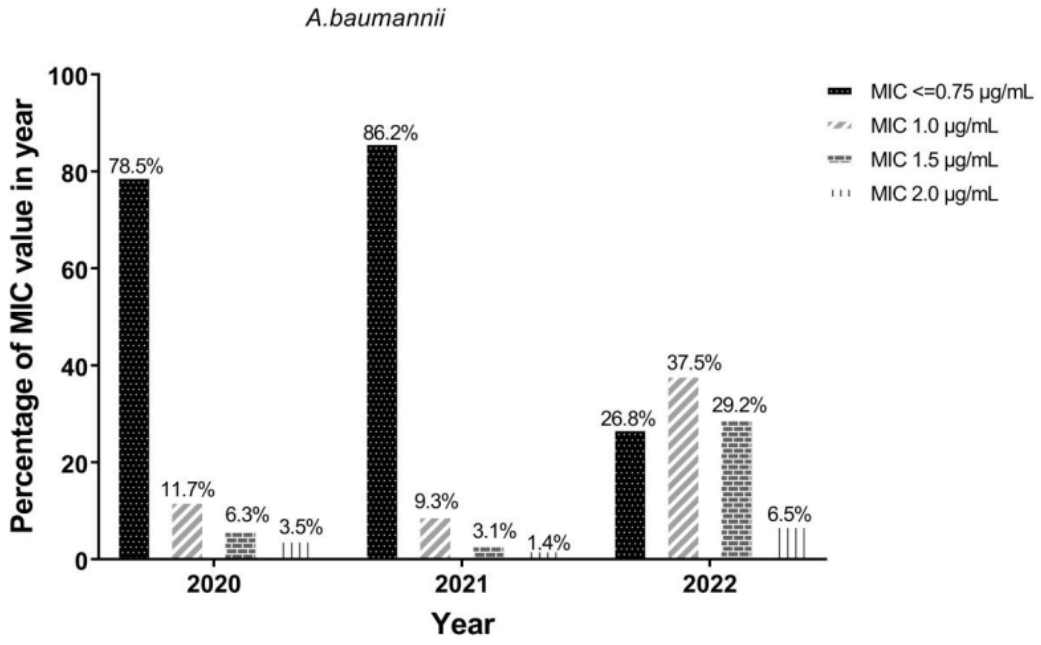

Figure 2. The trend of Colistin MIC values of *A. baumannii* from January 2020 to August 2022.

Table 3. The Colistin MIC value ranges of A.baumannii and P.aeruginosa

	MIC ≤ 0.75 μg/mL N (%)	MIC 1 μg/mL N (%)	MIC 1.5 μg/mL N (%)	MIC 2 μg/mL N (%)	p-value
A.baumannii	551 (70.8%)	126 (16.2%)	76 (9.8%)	25 (3.2%)	<0.001
P.aeruginosa	250 (60.0%)	103 (24.7%)	54 (12.9%)	10 (2.4%)	

During the period from January 2020 to August 2022, *A. baumannii* displayed a notable shift in Colistin MIC values, trending towards values close to 2 μg/mL. Specifically, MIC values ≤ 0.75 μg/mL were most prevalent in 2020 and 2021, while other MIC values were less common. By the first eight months of 2022, the proportions of MIC values at 1 μg/mL and 1.5 μg/mL increased, and the proportion of MIC values ≤ 0.75 μg/mL decreased significantly ($p<0.001$). Notably, the predominant MIC value of Colistin in 2022 was 1 μg/mL (37.5%) **(Figure 2).**

From January 2020 to August 2022, *P. aeruginosa* exhibited a changing trend in the common MIC values of Colistin, with a clear shift towards values close to 2.0 μg/mL. Among these, the MIC ≤ 0.75 μg/mL was most prevalent in 2020 and 2021 and showed a decreasing trend, with an increasing proportion of higher MIC values. By the first eight months of 2022, the MIC values at 1.0 μg/mL and 1.5 μg/mL increased significantly, and the proportion of MIC ≤ 0.75 μg/mL decreased sharply ($p < 0.001$). Notably, the prevalent MIC value in 2022 was 1 μg/mL (46.1%) ***(Figure 3)***.

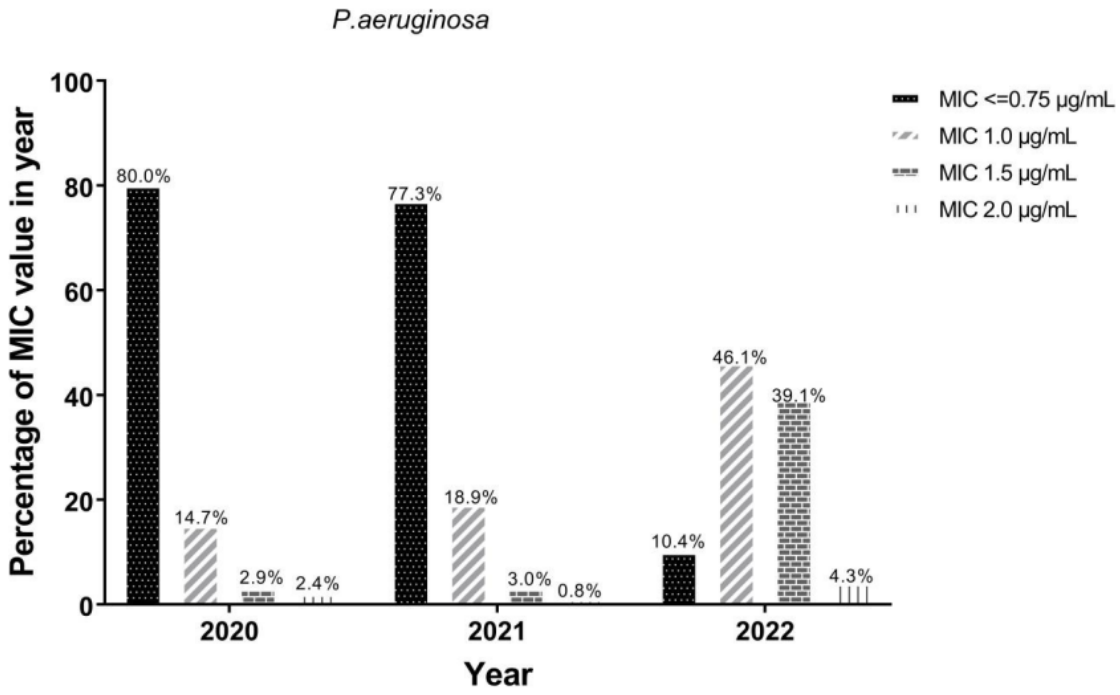

Figure 3. The trend of Colistin MIC values of *P.aeruginosa* from January 2020 to August 2022.

Carbapenem resistance characteristics based on Colistin MIC values

Colistin is considered the last-line antibiotic for treatment when *A. baumannii* and *P. aeruginosa* exhibit resistance to Carbapenem. Therefore, the Carbapenem resistance profile was compared based on the Colistin MIC values of *A. baumannii* and *P. aeruginosa*, providing information for the decision-making process when Colistin was used in the treatment of Carbapenem-resistant *A. baumannii* and *P. aeruginosa* **(Table 4)**. The Carbapenem resistance profile, based on Colistin Minimum Inhibitory Concentration (MIC) values of *A. baumannii*, did not exhibit statistically significant differences with two representative antibiotics, Meropenem and Imipenem. Regarding *P. aeruginosa*, the Carbapenem resistance profile based on Colistin MIC values did not differ significantly from Meropenem but differed from Imipenem ($p<0.001$). Specifically, Imipenem-resistant *P. aeruginosa* exhibited MIC values close to 2 μg/mL, higher than the non-resistant strain, with higher proportions of MIC values at 1 μg/mL and 1.5 μg/mL ($p<0.001$).

Table 4. Carbapenem resistance characteristics based on Colistin MIC values of A. baumannii and P. aeruginosa

Carbapenem resistance		Colistin MIC values					*p-value*
		MIC ≤ 0,75 µg/mL N (%)	MIC 1 µg/mL N (%)	MIC 1.5 µg/mL N (%)	MIC 2 µg/mL N (%)	Total N (%)	
A.baumannii							
Meropenem	Resistance	445 (72.0%)	98 (15.9%)	56 (9.1%)	19 (3.1%)	618 (100%)	*0.435*
	No resistance	33 (82.5%)	4 (10.0%)	3 (7.5%)	0 (0%)	40 (100%)	
Imipenem	Resistance	497 (69.8%)	118 (16.6%)	72 (10.1%)	25 (3.5%)	712 (100%)	*0.270*
	No resistance	43 (81.1%)	6 (11.3%)	4 (7.5%)	0 (0%)	53 (100%)	
P.aeruginosa							
Meropenem	Resistance	56 (54.4%)	30 (29.1%)	14 (13.6%)	3 (2.9%)	103 (100%)	*0.198*
	No resistance	165 (66.5%)	52 (21.0%)	25 (10.1%)	6 (2.4%)	248 (100%)	
Imipenem	Resistance	37 (40.7%)	32 (35.2%)	20 (22.0%)	2 (2.2%)	91 (100%)	*<0.001*

	No resistance	209 (65.5%)	69 (21.6%)	33 (10.3%)	8 (2.5%)	319 (100%)	

Discussion

Antibiotic resistance, especially in Gram-negative bacteria like *Enterobacteriaceae, Acinetobacter baumannii, and Pseudomonas aeruginosa*, poses a global health threat [11]. Colistin serves as a last-resort antibiotic against these strains, yet its efficacy is challenged by emerging drug-resistant strains, notably in *A. baumannii and P. aeruginosa* [12]. In this study, there was an increase in *A.baumannii* infections from 2020 to 2022, particularly during the peak of the COVID-19 pandemic in Vietnam, possibly due to factors such as prolonged hospitalizations and increased ventilator use. *A. baumannii* exhibited higher resistance rates to tested antibiotics compared to *P. aeruginosa*, particularly against fluoroquinolones and β-lactams. However, both bacterial species showed no resistance to Colistin during the study period.

The incidence of *A. baumannii* and *P. aeruginosa* infections is increasing each year. According to a systematic review by Usman Abubakar, during the COVID-19 pandemic, there was an increase in the incidence of carbapenem-resistant strains of *A. baumannii* and a decrease in *P. aeruginosa* [13]. These findings were consistent with results from the present study, which showed an increase in *A. baumannii* infections from 2020 to 2022, corresponding to the peak of the COVID-19 pandemic in Vietnam. This increase in *A. baumannii* infections was associated with prolonged hospitalizations, increased demand for ventilators, and the use of immunosuppressive drugs, particularly steroids [13]. These conditions could induce a favorable environment for the transmission of *A. baumannii* and *P. aeruginosa*, as they can persist on the surfaces of medical devices, especially ventilators.

The lower respiratory tract specimen, including sputum, bronchial wash, and endotracheal aspirate, is where *A. baumannii* and *P. aeruginosa* are most frequently isolated. *A. baumannii* and *P. aeruginosa* are

recognized as causative agents of hospital-acquired pneumonia and ventilator-associated pneumonia, with high multidrug resistance to commonly used antibiotics [14,15]. Patients with prolonged hospital stays and immunodeficiency, especially intensive care treatment, are at high risk of *A. baumannii* and *P. aeruginosa* infections. In addition, pus and urine also have a high positive rate for the two bacteria under consideration. These are specimens from primary infection sites where bacteria can penetrate deeper, leading to bloodstream infections and organ involvement [16].

The antibiotic resistance of *A. baumannii* and *P. aeruginosa* is highly complex, with a high resistance rate to commonly used antibiotics. This study observed a high antibiotic resistance rate of *A. baumannii*, exceeding 90%, against representative antibiotics in the Fluoroquinolone and β-Lactam groups. This result is consistent with the comprehensive report by Mutasim E Ibrahim on the high resistance rates of *A. baumannii* to these two antibiotic groups (Fluoroquinolone and β-Lactam) in hospitals in Saudi Arabia, ranging from 70-90% [17]. It indicates that *A. baumannii* is highly resistant to currently used antibiotics, necessitating the usage of last-line antibiotics such as Colistin fortreatment. By contrast, the *P. aeruginosa* strains in this study showed a lower resistance rate than *A. baumannii*, with approximately 30% of strains resistant to Carbapenem. This result is lower than the report of Jaime A Labarca in Latin American hospitals, in which resistance rates are 66% and 90% for *A. baumannii* and *P. aeruginosa* to Carbapenem, respectively [18]. This study demonstrates that the *A. baumannii* strains in the hospital are sensitive to Colistin. However, Dipti Pattnaik (2022) reported resistance rates of 2.8% and 1.4% for *A. baumannii* and *P. aeruginosa* to Colistin in the ICU, indicating an emerging resistance trend to Colistin for these bacteria [19]. This suggests that these bacterial strains are starting to increase resistance to Colistin. Therefore, in this study, we assess the trend of Colistin MIC values to predict the future resistance potential of the bacterial strains in the research.

The MIC values of Colistin for both *A. baumannii* and *P. aeruginosa* are predominantly in the range of ≤ 0.75 μg/mL. This result aligns with a study by Van Thi Khanh Nguyen (2021) on *P. aeruginosa* strains

isolated in Vietnam, which also showed that the majority of *P. aeruginosa* strains had MIC values for Colistin mainly concentrated at ≤ 0.75 µg/mL, with only 0.8% of strains resistant to Colistin [20]. In Federica Sacco's study (2021), *A. baumannii* strains also predominantly exhibited MIC values for Colistin at ≤ 0.75 µg/mL. This indicates that the use of Colistin for treating infections caused by *A. baumannii* and *P. aeruginosa* in hospitals still holds the potential for effective responses. Notably, *P. aeruginosa* strains with MIC values of 0.5 µg/mL or 1.0 µg/mL showed good clinical responses to Colistin treatment [20]. However, the concentration of MIC values for Colistin in *P. aeruginosa* strains, particularly near the cutoff point of 2 µg/mL, suggests that *P. aeruginosa* may be rapidly developing resistance to Colistin. Further research on the Colistin resistance genes of these bacteria is needed to understand this potential resistance development.

A.baumannii and *P.aeruginosa* are developing resistance to Colistin, as indicated by changes in Colistin MIC values from 2020 to 2022. The results show that both bacterial species have experienced variations in Colistin MIC values, with a concentration trend at MIC = 1 µg/mL and MIC = 1.5 µg/mL in 2022 ($p<0.001$). This suggests an increase in the bacteria's resistance to Colistin through a shift in MIC values, although these values are not classified as resistant. *A.baumannii* and *P.aeruginosa* resistance to Colistin primarily occurs through mechanisms such as alterations in outer membrane porins and reduced negative charge on phospholipid structures or increased expression of efflux pumps through known resistance genes [8]. However, in clinical practice in Vietnam, the detection of colistin-resistance genes in bacteria is not routinely performed as part of the diagnostic process. Therefore, the mechanisms behind the increased MIC values of Colistin in *A.baumannii* and *P.aeruginosa* in this study are unclear. Specialized studies on the resistance mechanisms of these two bacterial species are needed to develop management strategies and rational antibiotic use to prevent the emergence of Colistin-resistant strains in the future.

Colistin is considered one of the last-resort antibiotics for treating Carbapenem-resistant *A.baumannii* and *P.aeruginosa* [21]. Therefore, the differences in Colistin MIC values between strains with and without

Carbapenem resistance (Imipenem and Meropenem) are compared. There was no significant difference in Colistin MIC values between Carbapenem-resistant and non-resistant *A.baumannii* strains. However, Carbapenem-resistant *P.aeruginosa* strains exhibited higher percentages of Colistin MIC values at 1.0 μg/mL and 1.5 μg/mL compared to non-resistant strains. This difference may be related to the activity of non-specific efflux pumps causing multidrug resistance or may be due to another mechanism that is not clear [22].

The study's findings have important clinical implications. By identifying the upward trend in minimum inhibitory concentration (MIC) values for Colistin among *A. baumannii and P. aeruginosa* from 2020 to 2022, the study alerts healthcare providers to the potential development of resistance to Colistin in these bacterial strains. This information is crucial for guiding antibiotic treatment decisions, especially in cases of multidrug-resistant infections where Colistin is considered one of the last-resort antibiotics.

This study has some major strengths, demonstrating methodological rigor, ethical considerations, and providing valuable insights into antibiotic resistance trends. It makes a significant contribution to the field of antimicrobial resistance research in a Vietnamese context. Although the results of the present study are promising, there are some methodological limitations to bear in mind that should be addressed in future studies. This study was conducted retrospectively on antibiotic resistance data from patient samples collected at the hospital. Consequently, clinical information, such as the source of infection, length of hospital stays, and response to antibiotic treatment, was not gathered. It is not feasible to analyze the correlation between the sensitivity and resistance of bacteria with factors such as the age and gender of patients. Moreover, patients treated as inpatients at the hospital often underwent repeated testing. In addition, a tool for distinguishing between bacterial strains of the same species in different samples has not been approached. These limitations are acknowledged, and future, more comprehensive studies are needed to address these issues.

In future longitudinal studies, monitoring evolving trends in antibiotic resistance, including changes in MIC values over time, will provide insights into the dynamics of resistance development. Additionally, assessing clinical outcomes of patients treated with Colistin, considering factors such as treatment response, adverse effects, and long-term outcomes, is crucial. Furthermore, employing genomic analysis techniques to identify genetic determinants of antibiotic resistance in *A. baumannii* and *P. aeruginosa* strains will contribute to understanding the spread and evolution of resistance genes.

Conclusion

From 2020 to 2022, *A. baumannii* and *P. aeruginosa* showed an upward trend in MIC values for Colistin, nearing the threshold between susceptibility and resistance. While *P. aeruginosa* demonstrated a more pronounced change than *A. baumannii*, although it remained sensitive to Colistin. This suggests that these bacteria are gradually developing resistance to Colistin. Further advanced studies are needed to understand the resistance mechanisms of these bacteria, along with developing appropriate strategies for antibiotic management and usage before bacteria become completely resistant with Colistin.

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Declaration

Ethics approval

The research was accepted by the ethical principles established by the Ethics Committee for Biomedical Research at Nguyen Tri Phuong Hospital, as indicated by document number 746/NTP-HĐĐĐ, dated April 25, 2023.

Availability of data and material

The datasets are available from the corresponding author upon reasonable request.

Competing interests

The author(s) declare that they have no competing interests.

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Authors' contributions

- Conceptualization and methodology: THNN, HQN
- Software: HQN
- Validation: NTTL
- Formal analysis: HTNN, HCD
- Data curation: HMN
- Writing-original draft preparation: TNN
- Writing-review and editing: THNN, HMN
- Visualization: HMN
- Supervision: HMN
- Project administration: THNN, HMN

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