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Antimicrobial Resistance Trends in *Acinetobacter* spp. and *Pseudomonas* spp. Across the Pre-, Pandemic, and Post-COVID-19 Periods

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ABSTRACT

Introduction: The Coronavirus Disease-2019 (COVID-19) pandemic substantially changed healthcare delivery and may have influenced antimicrobial resistance (AMR) through increased empirical antibiotic prescribing, prolonged intensive care admissions, and extensive use of invasive interventions. As leading causes of healthcare-associated bloodstream infections, *Acinetobacter* spp. and *Pseudomonas* spp. represent important indicators for evaluating changes in resistance patterns over time. This study investigated temporal variations in AMR among these pathogens before, during, and after the COVID-19 pandemic.

Methods: A retrospective observational study was conducted at University of Health Sciences Türkiye, İstanbul Haseki Training and Research Hospital using blood culture isolates collected from January 2017 to December 2023. Adult hospitalized patients with non-fermentative Gram-negative bloodstream isolates were included. Antimicrobial susceptibility testing was interpreted according to the European Committee on Antimicrobial Susceptibility Testing breakpoints applicable at the time of analysis, and resistance profiles were compared across the pre-pandemic, pandemic, and post-pandemic periods.

Results: Overall, 740 *Acinetobacter* spp. and 268 *Pseudomonas* spp. isolates were evaluated. *Acinetobacter* spp. demonstrated a progressive increase in resistance to meropenem, colistin, gentamicin, and amikacin, with the highest resistance rates observed after the pandemic (all $p<0.001$). Meropenem resistance reached 98%, whereas colistin resistance increased to 32.2%. In contrast, *Pseudomonas spp.* exhibited significantly lower resistance to amikacin, gentamicin, ciprofloxacin, and imipenem during the post-pandemic period (all $p<0.05$), although the proportion of isolates with intermediate susceptibility increased for several antimicrobial agents.

Conclusion: Distinct changes in AMR patterns were observed following the COVID-19 pandemic, and these changes differed between bacterial species. The substantial increase in resistance among *Acinetobacter* spp., compared with the relatively favorable susceptibility profile of *Pseudomonas* spp., reinforces the need for ongoing local resistance surveillance, effective antimicrobial stewardship programs, and strengthened infection prevention measures.

Keywords: Antimicrobial resistance, gram-negative bacteria, bloodstream infection, COVID-19

Introduction

Bloodstream infections caused by Gram-negative pathogens rank among the most clinically significant healthcare-associated infections and present substantial therapeutic challenges because they are associated with elevated morbidity and mortality, particularly in critically ill patients. Among non-fermentative Gram-negative bacteria, *Acinetobacter* spp. and *Pseudomonas* spp. have a prominent role in hospital-acquired bloodstream infections owing to their ability to persist in the healthcare environment and to develop resistance to multiple antimicrobial agents (1-3).

The global expansion of antimicrobial resistance (AMR) has substantially complicated the management of infections caused by these pathogens. Several factors, including excessive antimicrobial consumption, prolonged hospitalization, invasive medical procedures, and persistent selective antimicrobial pressure, have accelerated the emergence and dissemination of resistant strains. As a result, increasing resistance to last-line therapeutic agents, particularly carbapenems and colistin, has become a major obstacle to effective treatment (4-6).

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The Coronavirus Disease-2019 (COVID-19) pandemic profoundly affected healthcare systems worldwide and introduced conditions that may have further influenced AMR patterns. Widespread empirical administration of broad-spectrum antibiotics, increased intensive care unit occupancy, prolonged hospital stays, and extensive use of invasive devices created favorable conditions for the selection and transmission of resistant microorganisms. Although the World Health Organization declared the end of the COVID-19 Public Health Emergency of International Concern on May 5, 2023, the consequences of these changes on AMR are likely to extend beyond the pandemic period (7,8).

Accumulating evidence indicates that the impact of the pandemic on AMR has not been uniform across bacterial species. Differences in ecological characteristics, resistance mechanisms, and responses to antimicrobial pressure may have resulted in distinct resistance trajectories among clinically important non-fermentative Gram-negative pathogens. Therefore, evaluating species-specific resistance trends is essential to better understand the long-term effects of the pandemic on hospital epidemiology (9,10).

Drawing on this reasoning, our working hypothesis was that the COVID-19 pandemic exerted differential effects on the resistance profiles *of Acinetobacter* spp. *and Pseudomonas* spp. To test this hypothesis, the current study sought to examine shifts in antimicrobial susceptibility among bloodstream isolates obtained across the pre-pandemic, pandemic, and post-pandemic timeframes at a tertiary-care hospital.

Methods

This retrospective observational study was conducted at University of Health Sciences Türkiye, İstanbul Haseki Training and Research Hospital Training and Research Hospital, a tertiary-care institution with a capacity of 700 inpatient beds, including 100 intensive care unit beds. The study period extended from January 2017 through December 2023. Hospitalized patients aged ≥18 years with blood cultures yielding non-fermentative Gram-negative bacteria were eligible for inclusion. To avoid duplicate sampling, only the first bloodstream isolate obtained from each patient was analyzed.

Among all non-fermentative Gram-negative organisms identified during the study period, only *Acinetobacter* spp. and *Pseudomonas* spp. were included because these species had complete microbiological data and sufficient isolate numbers for reliable statistical analysis.

The study protocol was approved by the University of Health Sciences Türkiye, İstanbul Haseki Training and Research Hospital Clinical Research Ethics Committee (decision number: 66-2024, approval date: August 29, 2024). As this is a retrospective study, informed consent was waived.

Patient characteristics, including age, sex, hospital ward, intensive care unit admission, bacterial species, and antimicrobial susceptibility results, were retrieved retrospectively from the Hospital Information Management System and the Laboratory Information System.

Blood culture bottles were processed using the automated BACTEC FX blood culture system (BD Diagnostics, USA). Positive cultures were subcultured; bacterial identification and antimicrobial susceptibility testing were then performed using the VITEK® 2 automated identification system (bioMérieux, France). Susceptibility interpretations were based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints that were applicable at the time each isolate was tested (11). Colistin susceptibility was determined by the reference broth microdilution method in accordance with current EUCAST recommendations. Isolates with incomplete or inaccurate microbiological records were excluded from the analysis.

For temporal comparisons, the study interval was divided into three predefined periods: the pre-pandemic period (January 2017-March 2020), the pandemic period (March 2020-December 2022), and the post-pandemic period (January 2023-December 2023).

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 28.0 (IBM Corp., Armonk, NY, USA). The distribution of continuous variables was evaluated with the Kolmogorov-Smirnov and Shapiro-Wilk tests. Continuous variables are presented as mean ± standard deviation, while median values were additionally provided in the tables to facilitate interpretation of data distribution. Categorical variables are expressed as frequencies and percentages.

Comparisons of categorical variables between study periods were performed using the chi-square test or Fisher's exact test when appropriate. Continuous variables were compared using the Kruskal-Wallis test. When statistically significant overall differences were identified, pairwise comparisons were conducted using the Mann-Whitney U test with Bonferroni correction. All statistical tests were two-sided, and a p value <0.05 was considered statistically significant.

Results

A total of 740 *Acinetobacter* spp. isolates were included in the study: 191 from the pre-pandemic period, 369 from the pandemic period, and 180 from the post-pandemic period. The mean age of patients differed significantly between periods ($p=0.007$). No statistically significant differences were found between periods with respect to gender distribution or patient admission location ($p>0.05$).

When antibiotic susceptibility results were evaluated, resistance rates to amikacin, colistin, gentamicin, and meropenem showed significant differences between periods ($p<0.001$). In particular, a marked increase in resistance rates to these antibiotics was observed in the post-pandemic period (Table 1).

When *Pseudomonas* spp. isolates were examined, 77 were evaluated in the pre-pandemic period, 113 in the pandemic period, and 78 in the post-pandemic period. The mean age of patients differed significantly between periods ($p<0.001$). In contrast, no significant differences were found between the periods regarding gender distribution and patient admission locations ($p>0.05$).

Analysis of antibiotic susceptibility results indicated that the resistance rates for amikacin, gentamicin, ciprofloxacin, and imipenem differed significantly between periods ($p<0.05$). Rates of resistance to these antibiotics decreased, particularly in the post-pandemic period. In contrast, no statistically significant differences were found between periods for ceftazidime and colistin ($p>0.05$) (Table 2).

Table 1. Demographic characteristics of patients with *Acinetobacter* spp. growth and antibiotic susceptibility of isolates

Variable	Pre-pandemic (n=191)	Pandemic period (n=369)	Post-pandemic (n=180)	p value/test
Age (mean ± SD)	68.4±18.7	64.7±16.6	66.7±16.8	0.007 (Kruskal-Wallis)
Median age	72.0	67.0	69.0	
Female, n (%)	86 (45.0%)	150 (40.7%)	72 (40.0%)	0.535 (chi-square)
Male, n (%)	105 (55.0%)	219 (59.3%)	108 (60.0%)	
Hospital ward, n (%)	44 (23.0%)	58 (15.7%)	31 (17.2%)	0.097 (chi-square)
ICU, n (%)	147 (77.0%)	311 (84.3%)	149 (82.8%)	
Amikacin-susceptible, n (%)	73 (38.2%)	118 (32.0%)	12 (6.7%)	p<0.001 (chi-square)
Amikacin-intermediate, n (%)	3 (1.6%)	1 (0.3%)	0 (0.0%)	
Amikacin-resistant, n (%)	115 (60.2%)	250 (67.8%)	168 (93.3%)	
Colistin-susceptible, n (%)	184 (96.3%)	353 (96.2%)	87 (59.6%)	p<0.001 (chi-square)
Colistin-intermediate, n (%)	0 (0.0%)	1 (0.3%)	12 (8.2%)	
Colistin-resistant, n (%)	7 (3.7%)	13 (3.5%)	47 (32.2%)	
Gentamicin-susceptible, n (%)	80 (41.9%)	88 (23.9%)	12 (9.4%)	p<0.001 (chi-square)
Gentamicin-intermediate, n (%)	0 (0.0%)	5 (1.4%)	0 (0.0%)	
Gentamicin-resistant, n (%)	111 (58.1%)	275 (74.7%)	116 (90.6%)	
Meropenem-susceptible, n (%)	38 (20.0%)	55 (14.9%)	3 (2.0%)	p<0.001 (chi-square)
Meropenem-intermediate, n (%)	1 (0.5%)	30 (8.2%)	0 (0.0%)	
Meropenem-resistant, n (%)	151 (79.5%)	283 (76.9%)	147 (98.0%)	

K: Kruskal-Wallis test (Mann-Whitney U for post-hoc comparisons), X^2: Chi-square test (Fisher's exact test when appropriate)
SD: Standard deviation, ICU: Intensive care unit

Table 2. Demographic characteristics of patients with *Pseudomonas* spp. growth and antibiotic susceptibility of isolates

Variable	Pre-pandemic (n=77)	Pandemic period (n=113)	Post-pandemic (n=78)	p value/test
Age (mean ± SD)	70.1±16.9	60.3±17.4	69.3±14.4	p<0.001 (Kruskal-Wallis)
Median age	70.0	64.0	71.0	
Female, n (%)	32 (41.6%)	42 (37.2%)	34 (43.6%)	0.650 (chi-square)
Male, n (%)	45 (58.4%)	71 (62.8%)	44 (56.4%)	
Hospital ward, n (%)	19 (24.7%)	31 (27.4%)	31 (39.7%)	0.087 (chi-square)
ICU, n (%)	58 (75.3%)	82 (72.6%)	47 (60.3%)	
Amikacin-susceptible, n (%)	21 (29.2%)	46 (51.7%)	63 (90.0%)	p<0.001 (chi-square)
Amikacin-intermediate, n (%)	3 (4.2%)	1 (1.1%)	0 (0.0%)	
Amikacin-resistant, n (%)	48 (66.7%)	42 (47.2%)	7 (10.0%)	
Colistin-susceptible, n (%)	69 (95.8%)	84 (94.4%)	36 (72.0%)	0.204 (chi-square)
Colistin-intermediate, n (%)	0 (0.0%)	0 (0.0%)	8 (16.0%)	
Colistin-resistant, n (%)	3 (4.2%)	5 (5.6%)	6 (12.0%)	
Gentamicin-susceptible, n (%)	31 (41.3%)	51 (51.0%)	13 (86.7%)	0.005 (chi-square)
Gentamicin-intermediate, n (%)	0 (0.0%)	3 (3.0%)	0 (0.0%)	
Gentamicin-resistant, n (%)	44 (58.7%)	46 (46.0%)	2 (13.3%)	
Ceftazidime-susceptible, n (%)	12 (52.2%)	30 (49.2%)	29 (39.7%)	0.280 (chi-square)
Ceftazidime-intermediate, n (%)	0 (0.0%)	13 (21.3%)	17 (23.3%)	
Ceftazidime-resistant, n (%)	11 (47.8%)	18 (29.5%)	27 (37.0%)	
Ciprofloxacin-susceptible, n (%)	19 (25.3%)	20 (20.6%)	10 (13.2%)	p<0.001 (chi-square)
Ciprofloxacin-intermediate, n (%)	0 (0.0%)	29 (29.9%)	46 (60.5%)	
Ciprofloxacin-resistant, n (%)	56 (74.7%)	48 (49.5%)	20 (26.3%)	
Imipenem-susceptible, n (%)	20 (27.0%)	26 (26.5%)	9 (13.0%)	0.008 (chi-square)
Imipenem-intermediate, n (%)	0 (0.0%)	15 (15.3%)	27 (39.1%)	
Imipenem-resistant, n (%)	54 (73.0%)	57 (58.2%)	33 (47.8%)	

K: Kruskal-Wallis test (Mann-Whitney U for post-hoc comparisons), X^2: Chi-square test (Fisher's exact test when appropriate)
SD: Standard deviation, ICU: Intensive care unit

Discussion

This study revealed that the AMR profiles of non-fermentative Gram-negative bloodstream isolates underwent notable shifts across the pre-pandemic, pandemic, and post-pandemic timeframes. While *Acinetobacter* spp. exhibited a substantial increase in resistance to several last-line antimicrobial agents, *Pseudomonas* spp. showed more variable susceptibility profiles, with reduced resistance to selected antibiotics. These findings indicate that the impact of the COVID-19 pandemic on AMR differed between these two major non-fermentative Gram-negative pathogens.

The clinically significant escalation in resistance among *Acinetobacter* spp., particularly to meropenem and colistin, is concerning because these agents constitute critical therapeutic pillars for managing severe nosocomial bloodstream infections. Several factors associated with the pandemic may have contributed to this increase, including the widespread empirical use of broad-spectrum antibiotics, prolonged stays in intensive care units, more frequent invasive procedures, and disruptions in antimicrobial stewardship and infection prevention practices. Moreover, the well-established ability of *Acinetobacter* spp. to survive in the hospital environment and to readily acquire resistance mechanisms may have promoted the persistence and dissemination of multidrug-resistant strains during and after the pandemic.

Previous studies have identified *Acinetobacter* spp. and *Pseudomonas* spp. as the predominant non-fermentative Gram-negative pathogens causing hospital-acquired bloodstream infections, particularly in intensive care units. The EUROBACT-2 and VIRAPERU studies also reported high carbapenem resistance rates among *Acinetobacter* isolates and highlighted the considerable burden of multidrug-resistant Gram-negative bacteria in critically ill patients (12,13). Similarly, Müderris et al. (14) reported that *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were among the most frequently isolated pathogens causing superinfections in patients with COVID-19, whereas Özçelik et al. (15) identified these microorganisms as the predominant non-fermentative Gram-negative isolates recovered from blood cultures of intensive care unit patients. Our findings are consistent with these reports; however, they further demonstrate that AMR evolved differently in the two species over time. Specifically, resistance increased markedly in *Acinetobacter* spp. during the post-pandemic period, whereas *Pseudomonas* spp. showed relatively stable or even reduced resistance to several antimicrobial agents.

The effects of the COVID-19 pandemic on resistance patterns have been similarly documented in the literature. Etiz et al. (16) and Araj et al. (17) both noted a rise in resistance among hospital-acquired Gram-negative organisms during and following the pandemic, attributing these trends to modifications in antibiotic prescribing behaviors and the overwhelming strain placed on healthcare infrastructure. Our results agree with these observations but also suggest that the magnitude of these changes may vary by bacterial species. The pronounced increase in resistance among *Acinetobacter* spp., together with a more heterogeneous response observed in *Pseudomonas* spp., indicates that the ecological consequences of the pandemic were not uniform across non-fermentative Gram-negative pathogens.

A systematic review focusing on *Pseudomonas aeruginosa* bloodstream infections among COVID-19 patients highlighted extended ICU stays, dependence on mechanical ventilation, and broad-spectrum antibiotic exposure as key predisposing factors in critically ill individuals (18). Although our findings confirm the continued clinical importance of *Pseudomonas* spp. in this setting, the reduction in resistance observed for several antimicrobial agents during the post-pandemic period suggests that antimicrobial stewardship practices, infection control measures, or changes in patient characteristics may have influenced resistance patterns differently from those observed in *Acinetobacter* spp. This underscores the importance of conducting pathogen-specific analyses rather than grouping all non-fermentative Gram-negative bacteria together when evaluating resistance dynamics.

Overall, our findings indicate that the COVID-19 pandemic was associated with species-specific changes in AMR among non-fermentative Gram-negative bloodstream isolates. The marked increase in resistance observed in *Acinetobacter* spp., particularly against last-line antimicrobial agents, highlights the ongoing need for local resistance surveillance, effective antimicrobial stewardship, and robust infection prevention strategies to preserve the efficacy of currently available treatment options.

Study Limitations

This study has several limitations. First, the study was conducted at a single center, and the findings may not be generalizable to other hospitals and healthcare settings. Second, due to the retrospective design of the study, data on some clinical variables may be limited, and potential information bias may have occurred. Furthermore, the lack of detailed antibiotic consumption data for the study period limited the direct assessment of the relationship between antibiotic use and AMR trends. Finally, the lack of analysis of parameters such as mortality, length of hospital stay, or clinical outcomes limited the assessment of the clinical impact of the observed resistance changes.

Another limitation of this study is the unequal duration of the study periods. While the pre-pandemic and pandemic periods covered several years, the post-pandemic period included only one year (2023). Therefore, the findings related to the post-pandemic period should be interpreted with caution, as the shorter observation period may have influenced the observed AMR trends.

Changes in EUCAST breakpoint definitions and the reclassification of the "I" category as "Susceptible, increased exposure" may have influenced susceptibility categorization across the study period.

Conclusion

The COVID-19 pandemic was associated with pathogen-specific changes in AMR among non-fermentative Gram-negative bloodstream isolates. While *Acinetobacter* spp. demonstrated a marked post-pandemic increase in resistance to several last-line antimicrobial agents, *Pseudomonas* spp. exhibited more favorable susceptibility trends to selected antibiotics. These observations underscore that the pandemic's influence on resistance was not homogeneous across bacterial species and reinforce the critical role of sustained local surveillance,

antimicrobial stewardship initiatives, and robust infection prevention protocols in guiding empirical treatment decisions and safeguarding remaining therapeutic options.

Ethics

Ethics Committee Approval: The study protocol was approved by the University of Health Sciences Türkiye, İstanbul Haseki Training and Research Hospital Clinical Research Ethics Committee (decision number: 66-2024, approval date: August 29, 2024).

Informed Consent: As this is a retrospective study, informed consent was waived.

Footnotes

Authorship Contributions: Concept - E.Z., M.K.T., H.T., H.E., Ü.A.; Design - E.Z., H.T., H.E., Ü.A.; Data Collection or Processing - M.K.T., H.T.; Analysis or Interpretation - H.T.; Literature Search - E.Z., M.K.T., H.E., Ü.A.; Writing - E.Z., M.K.T.

Conflict of Interest: No conflict of interest was declared by the authors.

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