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Species distribution and resistance profile of clinical isolates from an intensive care unit during pre- and post-COVID-19 pandemic periods

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ABSTRACT

Background: The increasing incidence of antibiotic resistance was a major health problem before the Corona Virus Disease 2019 (COVID-19) pandemic, but in the post-COVID era this problem has worsened. The purpose of this study is to describe and analyze the incidence of multidrug-resistant pathogens in an intensive care unit before (2019), and at the end of the COVID-19 pandemic (2022).

Methods: This is a retrospective, single-center, observational cohort study of infections in patients admitted to the Intensive Care Unit (ICU) of the Sf. Spiridon County Emergency Hospital (Iasi, Romania) during two distinct periods- before and after the first three waves of the COVID-19 pandemic. Species distribution and resistance profile of clinical isolates were recorded.

Results: Among the isolated pathogens, the Gram-negative bacteria strains accounted for the majority of microorganisms in both years (77.81% in 2019 and 71.01% in 2022) with the Acinetobacter baumannii isolates being the most abundant in 2019 (87 strains, 32.22%), and Klebsiella pneumoniae in 2022 (107 strains, 40.07%). When considering the overall profile of resistance rates, significant post-pandemic increases were observed for the following antimicrobial agents: tobramycin, ceftazidime, ertapenem, colistin, piperacillin/tazobactam and also for the recently introduced antibiotics in Europe- ceftolozane-tazobactam, ceftazidime-avibactam, imipenem-relebactam.

Conclusions: We have noticed the emergence of new multidrug resistant strains in the ICU. In ICUs, the increase in antibiotic resistance following the COVID-19 pandemic is a multifaceted issue driven by the interplay of clinical practices, healthcare system disruptions, and infection control challenges. It was fueled by several factors like overuse and misuse of antibiotics, extended hospital stays, and inadequate infection control.

Keywords: antibiotic resistance, COVID-19 pandemic, healthcare-associated infections

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INTRODUCTION

The COVID-19 pandemic had a negative impact globally in all socio-economic fields. A particular long-term effect was found in the field of resistance of pathogens to antibiotics. Several reports have highlighted the changing epidemiology of microbial agents in the intensive care unit (ICU) during the different waves of the COVID-19 pandemic, and in particular the significant increase of multidrug-resistant *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterococcus* spp in the ICU [1].

The increasing incidence of antibiotic resistance was a major health problem before the COVID-19 pandemic, but this problem has worsened in the post-COVID era. Many of the patients with severe COVID-19 infection in

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ICU required ventilatory support, central venous catheterization, urinary catheter, which led to the increasing the risk of infections associated with medical devices including ventilator-associated pneumonia, urinary tract infections, bloodstream infections, and catheter-associated infections. Despite the rigorous epidemiological measures to isolate and limit the transmission of the COVID-19 infection, patients with severe COVID-19 pneumonia presented bacterial infections, pre-hospitalization antibiotic therapy, the use of immunosuppressive therapy, the uncontrolled/overuse of broad-spectrum antimicrobials drug with multiple drug combinations administered to patients in hospitals and ICUs, prolonged periods of hospitalization, factors that led to a selection pressure of multidrug-resistant microbial agents and to the change of the antibiotic resistance profile of pathogenic agents [2,3]. The purpose of this study is to describe and analyze the incidence of multidrug-resistant microbial pathogens in an ICU before the COVID-19 pandemic (in 2019), and at the end of the COVID-19 pandemic (in 2022). We initiated this study based on the hypothesis that the strict epidemiological measures implemented during the first wave of the COVID-19 pandemic would also lead to a decrease in the incidence of healthcare-associated infections, which are typically caused by multidrug-resistant strains in the ICU.

METHODS

The studies involving human participants were reviewed and approved by the Institutional Ethics Committee of St. Spiridon County Clinical Emergency Hospital of Iasi in 2025.

This is a retrospective, single-center, observational cohort study of patients admitted to the ICU of Sf. Spiridon County Emergency Hospital (Iasi, Romania) during two distinct periods - before and after the first three waves of the COVID-19 pandemics (i.e. January to December 2019, and January to December 2022, respectively). The hospital is the biggest tertiary healthcare unit in the region having a total number of 1000 beds. The ICU has 25 beds that serve several units – mainly internal medicine and surgery cases. The bed turnover rate was 318.6 in 2019, and 273.8 in 2022, respectively. The inclusion criteria were: age higher than 18-year-old, patients hospitalized in ICU for healthcare-associated infections and other conditions, bacterial or fungal non-repetitive isolates with clinical significance. Exclusion criteria included: isolates from patients without clinical signs of infection or lack of inflammatory reaction on Gram-stained smears; repetitive isolates from the same patient and same type of sample; isolates from rectal swabs and pharyngeal exudates during regular epidemiological screening. Species identification was done using the MALDI-TOF MS equipment Biotyper (Brucker, Germany). Antimicrobial susceptibility testing was performed using the broth microdilution method (Micronaut, USA) and the obtained MICs were interpreted following the EUCAST recommendations. The resistant isolates were classified as multidrug resistant (MDR), extensively drug resistant (XDR), and pan-drug resistant (PDR) according to ECDC rules [4].

Specific statistical parameters were calculated for each microbial species and various specimens, and for each microbial species and antimicrobial agent, respectively. Two-tailed *p*-values were calculated and $p<0.05$ was considered statistically significant.

RESULTS

General characteristics of samples

A total of 3799 samples were collected from 7965 patients admitted to the ICU during the year before the COVID-19 pandemics (January to December 2019). A lower number of samples (n=3404) was collected from 6846 patients during the year following the first 3 waves of pandemics (January to December 2022). Among the investigated specimens, the percentage of positive bacterial cultures was similar between 2019 and 2022 with no significant differences (9.14% in 2019 vs. 11.05% in 2022; $p=0.1806$; OR=0.8095). Additionally, no statistical differences emerged from analyzing the percentage of strains isolated from the sample types comprising urine - 74 strains identified (21.33%) in 2019 vs. 102 strains (27.13%) in 2022 ($p=0.0826$; OR=0.7281); wound specimens- 75 strains (21.61%) in 2019 vs. 95 strains (25.27%) in 2022 ($p=0.2251$; OR=0.8156); blood and central venous catheter insertions - 59 (17.00%) in 2019 vs. 72 (19.15%) in 2022 ($p=0.4990$; OR=0.8650). Interestingly, from endotracheal aspirates, 127 strains were isolated in 2019 (36.6%), significantly more than in 2022- 73 strains (19.41%) ($p<0.0001$; OR=0.2396), while a lower number of strains was isolated from drainage fluid specimens in 2019 compared to 2022 - 12 strains (3.46%) in 2019 vs. 34 strains (9.04%) in 2022 ($p=0.0021$; OR=0.3603).

Distribution of pathogens

We categorized the ICU infections as healthcare associated infections (HAIs: infections onset occuring ≥ 48 hours after hospital admission and/or attributed to hospital medical care, procedures or devices; clinical signs and/or laboratory-confirmed infection not present or incubating at the time of hospital admission) and non-HAIs, and compared the differences observed between the two investigated periods, before and after two years of pandemics while stringent epidemiological methods were implemented. While no differences between the total number of HAIs or non-HAIs were observed between the two selected years (HAI/ non-HAI ratio of 8.1 in 2019 and 10.6 in 2022), the general resistance rate, defined as the percentage of patients with HAI or non-HAI infections was higher in 2022 than 2019 (5.9% vs. 4.7%, *p*=0.0004). Regarding the distribution of non-MDR, MDR and XDR among non-HAI infections, no differences were observed between the two years. In contrast, both the number and proportion of MDR infections increased, while XDR infections showed a significant decrease in 2022 compared to 2019 (*p*=0.0004).

Among the isolated pathogens, the Gram-negative bacteria (GNB) strains accounted for the majority of microorganisms in both years, with a higher percentage in 2019 (77.81% in 2019 and 71.01% in 2022; *p*=0.0410; OR = 1.431). Importantly, among GNB, the *Acinetobacter baumannii* isolates were the most abundant in 2019 (87 strains, 32.22%), while *Klebsiella pneumoniae* outweighed in 2022 (107 strains, 40.07%). The higher number of *Acinetobacter baumannii* isolates observed in 2019 originated from endotracheal aspirates (47 strains, 54.03% in 2019 vs. 18 strains, 36.73% in 2022; *p*=0.0734; OR=2.024), and the higher number of *Klebsiella pneumoniae* noted in 2022 emerged from urine (21 strains, 19.63% in 2022 vs. 3 strains, 6.81% in 2019; *p*=0.0537; OR=3.337) and wound (40 strains, 37.38% in 2022 vs. 12 strains, 27.27% in 2019; *p*=0.2630; OR=1.592) specimens. *Pseudomonas aeruginosa* isolates occupied the second place as frequency among GNB in both years (25.56% in 2019 vs. 24.35% in 2022; *p*=0.7655; OR=1.067). Interestingly, the number of *P. stuartii* and other Enterobacterales (*Enterobacter kobei*, *Enterobacter cloacae*, *Proteus mirabilis*, *Proteus vulgaris*) isolates was lower in 2022. Regarding the Gram-positive pathogens, the enterococci group accounted for the majority of bacteria in both years (27.27% in 2019 and 26.61% in 2022; *p*=1.000; OR=1.034). Importantly, we also observed a limited number of *Staphylococcus aureus* strains and a high number of *Candida* spp. isolates in both years.

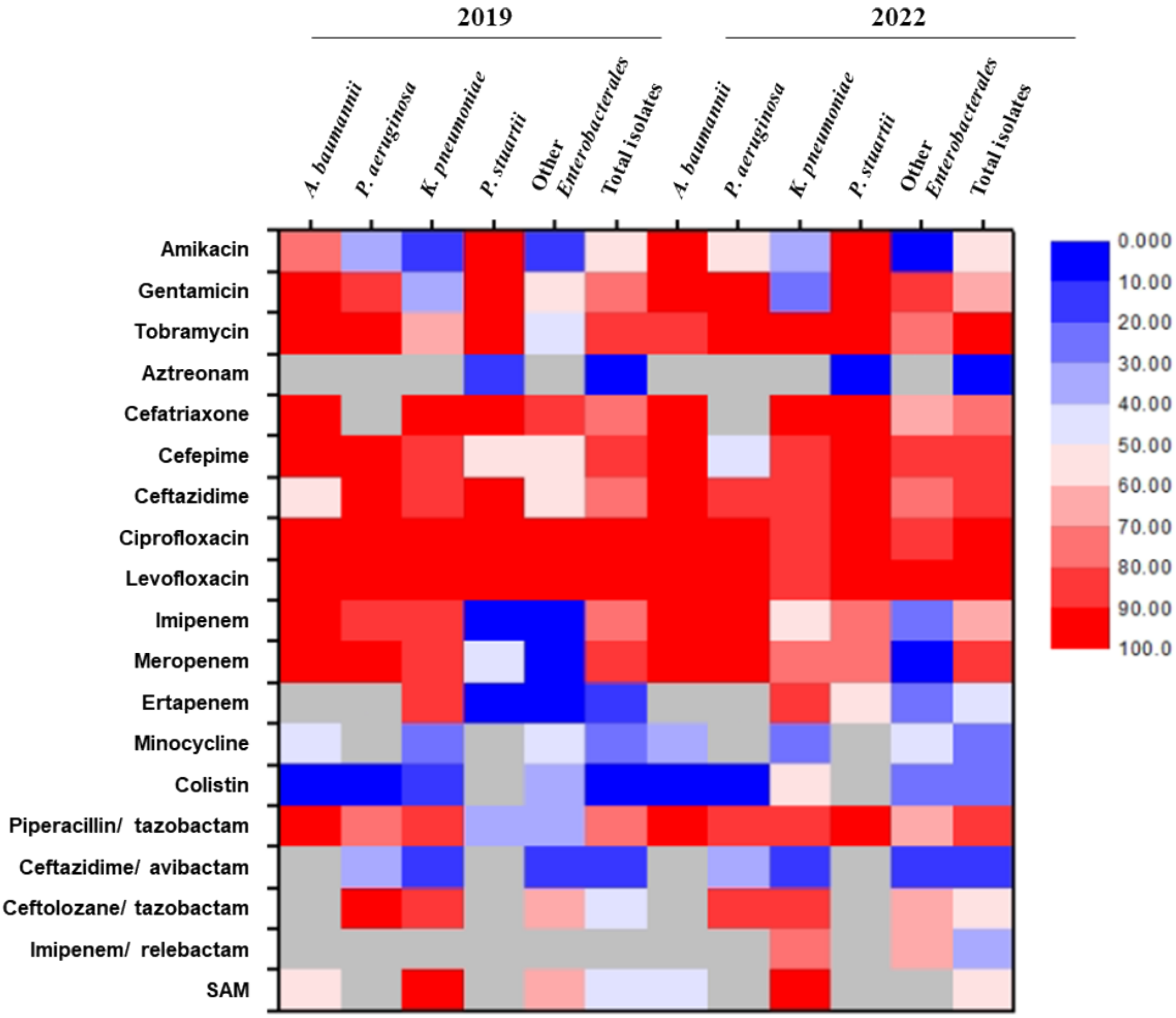

Figure 1. Resistance (%) to various antibiotics for bacterial strains isolated in ICU during 2019 and 2022
The heat map of resistance rates displays values below 50% in blue, while values exceeding 50% are highlighted in red.

Drug resistance

A comparative overview (2019 vs. 2022) on antibiotic resistance for the clinical isolates is represented in the Figure 1. When considering the overall profile of resistance rates, significant increases were observed when analyzing the following antimicrobial agents: tobramycin, ceftazidime, ertapenem, colistin, piperacillin/tazobactam and also the recently introduced antibiotics in Europe ceftolozane-tazobactam, ceftazidime-avibactam, imipenem-relebactam (Figure 2). Additionally, a slight decrease of resistence rate was noticed for gentamicin due to a reduction in the resistance rate to this antibiotic of *K. pneumoniae* isolates.

A. baumannii

The resistant rates of *A. baumannii* to amikacin and ceftazidime significantly increased in 2022 compared to 2019, with a relative ratio (RR) of 1.35 (p=0.0006) and 1.87 (p<0.0001), respectively. The resistance rate to minocycline and sulbactam-ampicillin (SAM) tended to slightly decrease from 2019 to 2022, however with no significant differences (from 48.75% to 32.65% for minocycline, and from 55% to 42.8% for SAM). Importantly, the resistance rate to colistin was 0%, while for the other tested antimicrobial agents over 90% in both years (Gentamicine, Ceftriaxone, Cefepime, Ciprofoxacin, Levofloxacin, Meropenem, Imipenem).

P. aeruginosa

The resistance rates of *P. aeruginosa* to cefepime - a fourth generation cephalosporine, significantly decreased by almost 50%, from 93.62% in 2019 to 45.45% in 2022 (p<0.0001). Similarly, the resistance rate to ceftazidime, a third generation cephalosporine, decreased from 93.62% in 2019 to only 81.82% in 2022. The resistance rates to amikacin, imipenem, and piperacillin/tazobactam tended to slightly increase by 10-15% from 2019 to 2022. Among the new class of antimicrobial agents reported to have a good antibacterial activity against *P. aeruginosa*, only ceftazidime/avibactam proved to be an efficient therapeutic alternative (the resistance rates were 38.3% in 2019 and 36.36% in 2022). Additionally, *P. aeruginosa* isolates were generally resistant to ceftolozane/tazobactam, quinolones, and aminoglycosides (gentamicin, tobramycin).

K. pneumoniae

The antibiotics that showed a good antibacterial activity against *K. pneumoniae*, associated with resistance rates below 60% in both years, included aminoglycosides (amikacin, gentamicin), minocycline, colistin, and ceftazidime/avibactam. However, the resistance rates of *K. pneumoniae* to amikacin and colistin markedly increased in 2022 compared to 2019 (RR 1.93, p=0.0361, and RR 2.99, p=0.0002, respectively). The resistance rate to tobramycin also significantly increased from 2019 to 2022 (from 68.3% to 91.8%, p=0.0004). Of interest, since the resistance rate to imipenem significantly decreased from 82.93% in 2019 to 51.02% in 2022 (p=0.0005), this antimicrobial agent was reintroduced in the second-line of therapeutic agents efficient against MDR *K. pneumoniae* isolates.

P. stuartii

Despite a lower number of *P. stuartii* isolates in 2022, the resistance rates to all tested carbapenems dramatically increased above 50%. For instance, the resistance rate to imipenem significantly increased from only 9.1% in 2019 to 71.4% in 2022 (p=0.0002), and to ertapenem from 4.5% to 50% (p=0.0026). The resistance

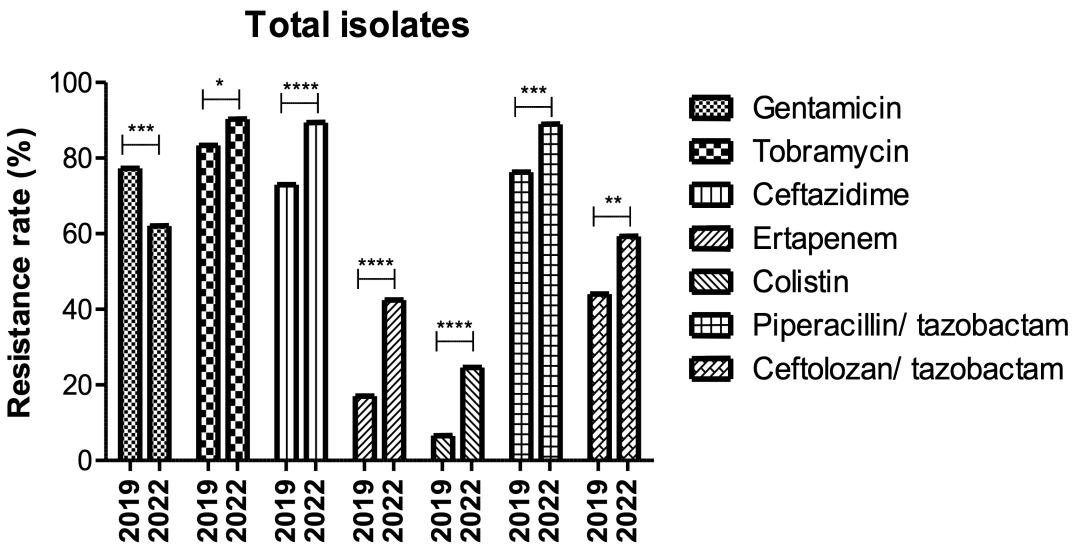

Figure 2. Overall resistance rates to specific antibiotic drugs in 2019 and 2022
Chi-squared test was applied. Significance: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001

rate to meropenem, initially higher than for the other 2 carbapenems, increased from 40.9% in 2019 to 78.6% in 2022 (p=0.0407). Importantly, while the resistance rates to cefepime and piperacillin/tazobactam were below 60% in 2019, all the *P. stuartii* isolates from 2022 were XDR, being insensitive also to these antimicrobial agents. Thus, the only available therapeutical agent that remained was aztreonam, to which all the strains were sensitive in 2022 (resistance rate 0%) compared to 2019 (resistance rate 18.18%). These evident changes observed in the profile of resistance rates for *P. stuartii* to various antimicrobial agents suggests an evolution from XDR towards PDR strains.

Other *Enterobacterales* (*E. kobei, E. cloacae*, *P. mirabilis*, *P. vulgaris*)

When analyzing the other *Enterobacterales* isolates, the resistance rates to five antimicrobial agents significantly increased from 2019 to 2022. Importantly, the resistance rate to aminoglycosides (gentamicin and tobramycin) and cefepime, initially 42-52% in 2019 raised to 78-86% in 2022. While all the strains were sensitive to imipenem in 2019, 21.4% of isolates became resistant in 2022. Additionally, the percentage of isolates resistant to piperacillin/tazobactam doubled in 2022 (raising from 33.3% in 2019 to 64.3% in 2022).

MDR Gram-positive bacteria

The isolates of enterococci and *S. aureus* represented a small percentage of the total MDR strains isolates in the ICU (6.74% in 2019 and 8.33% in 2022). All the *S. aureus* isolates were susceptible to vancomycin and teicoplanin, while the potential therapeutic agents against enterococci remained linezolid (resistance rates 42.8% in 2019 and 37.5% in 2022) and tigecycline (resistance rates 66.7% in 2019 and 40.6% in 2022).

Candida species

For *Candida* yeasts, the most frequent isolates are related to urine samples. The resistance rate to fluconazole was low in both years for isolates belonging to other species (*Candida albicans*, *Candida tropicalis*, *Candida kefir*, *Candida dubliniensis*) than *C. krusei* and *C. glabrata*: 10% versus 5.4% (2019 vs. 2022).

DISCUSSION

The COVID-19 pandemic has significantly impacted global health systems and patient care practices, leading to unintended consequences on healthcare-associated infections (HAIs): an increase in their frequency and a higher antibiotic resistance rate. This phenomenon arises from multiple factors intertwined with the dynamics of the pandemic, reflecting both the direct and indirect influences on antimicrobial stewardship. Different studies performed in various countries examined the impact of prevention and control measures implemented during the COVID-19 pandemic on the prevalence of HAIs. Some of them found a significant decrease in HAIs prevalence during and post-COVID-19 compared to pre-COVID-19 rates in most hospital departments excepting ICU [5,6]. Other studies highlighted a significant increase of HAIs during COVID-19 – a fact that is mainly related to the local, regional, and national specific conditions occurring during the period mentioned [7,8].

In our study, no significant differences occurred between the pre- and post-COVID-19 periods concerning the prevalence of HAIs despite the fact that the ratio HAIs/non-HAIs was higher in 2022. Contrarily, an increase in the resistance rate of some bacterial species to particular antibiotics has been demonstrated – situation also reported by other studies [7,9,10]. This is mainly related to the overuse of antibiotics in COVID-19 patients worldwide to treat bacterial associated infections or as preventive measure [11], including Romania [12-14]. One study examined the antibiotic resistance profiles in the ICU, comparing the pre- and post-pandemic periods in our country [10]. The most frequently isolated pathogens in both periods were *S. aureus* and *Klebsiella* spp. After the COVID-19 pandemic, an increase in *A. baumannii* and *P. aeruginosa* isolates was observed, while *Klebsiella* spp. isolation rates decreased. In our study, Gram-negative bacilli (GNB) were the most frequently isolated microorganisms. *A. baumannii* ranked first in 2019, whereas *K. pneumoniae* became predominant in 2022. In both periods, *P. aeruginosa* ranked second.

Regarding antibiotic resistance patterns for *Klebsiella* spp., both studies reported a significant increase in resistance to colistin (from 0.38% to 20.51%, RR 1.93, p=0.0361) and aminoglycosides (gentamicin in their study: from 44.62% to 64.85%; amikacin and tobramycin in our study: RR 2.99, p=0.0002, and from 68.3% to 98.8%, p=0.0004, respectively). We did not identify any *S. aureus* strains resistant to teicoplanin, whereas according to the other study, resistance increased from 26.31% to 53.40% [10]. *A. baumannii* showed a significant increase in resistance to amikacin and ceftazidime (RR 1.35, p=0.0006, and RR 1.87, p<0.0001, respectively). Interestingly, no resistance to colistin was recorded in our study, whereas resistance rates for other antimicrobials (gentamicin, ceftriaxone, cefepime, ciprofloxacin, levofloxacin, meropenem, imipenem) exceeded 90% in both years. Golli et al. [10] reported a post-COVID-19 increase in *Acinetobacter* spp. isolates, along with a rise in colistin resistance (from 3.37% to 18.09%, p<0.001). For *P. aeruginosa*, the resistance rate to cefepime decreased substantially, from 93.62% in 2019 to 45.45% in 2022 (p<0.0001). Similar trends were noted in the other study (from 76.47% to 58.51%, p=0.01) [10]. Among the newer antimicrobial agents, ceftazidime/avibactam demonstrated promising activity against *P. aeruginosa*, with resistance rates of 38.3% in 2019 and 36.36% in 2022. However, *P. aeruginosa* isolates generally exhibited high resistance to ceftolozane/tazobactam.

However, our study has several limitations, including the absence of data on the bed turnover rate, as well as limited information regarding the general characteristics of the patients and the presence of comorbidities. These factors could have provided a more comprehensive understanding of the patient population and potential confounding variables influencing infection rates and antimicrobial resistance.

To our knowledge, this is the first study in Romania to examine potential changes in antimicrobial resistance before and after the COVID-19 pandemic for the newly introduced antibiotics in Europe (ceftolozane-tazobactam, ceftazidime-avibactam, imipenem-relebactam). Overall, significant post-pandemic increases in resistance were observed for tobramycin, ceftazidime, ertapenem, colistin, piperacillin/tazobactam, as well as ceftolozane-tazobactam, ceftazidime-avibactam, and imipenem-relebactam.

CONCLUSIONS

From the comparative data analysis over the two years, we observed the emergence of new MDR strains of *K. pneumoniae*, *P. aeruginosa*, and *P. stuartii*. Statistical analysis did not reveal significant differences in antibiotic consumption that could account for the increased resistance to the antibiotics used. Therefore, we can conclude that the observed changes are more likely due to the emergence of new strains rather than an increase in resistance driven by antibiotic selection pressure. Unfortunately, our study could not confirm the genetic relationship between MDR strains isolated before and after the COVID-19 pandemic, as genetic analyses were not performed.

The increase in antibiotic resistance following the COVID-19 pandemic is a multifaceted issue driven by the interplay of clinical practices, healthcare system disruptions, and infection control challenges. Several factors have fueled the increase in antibiotic resistance in ICUs post-COVID-19: overuse and misuse of antibiotics; extended hospital stays; inadequate infection control.

Addressing this issue requires a concerted effort to strengthen antimicrobial stewardship, enhance diagnostic capabilities, and ensure effective infection prevention and control measures. As the world recovers from COVID-19, it is crucial to apply the lessons learned to mitigate the impact of antibiotic resistance and safeguard the efficacy of these vital medications for future generations.

ABBREVIATIONS

COVID-19 – Corona Virus Disease 2019

ECDC – European Centre for Disease Prevention and Control

GNB – Gram Negative Bacteria

HAI – Healthcare Associated Infection

ICU – Intensive Care Unit

MDR – Multidrug Resistant

PDR – Pan-drug Resistant

SAM – Sulbactam/Ampicillin

XDR – Extensively Drug Resistant

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None.

AUTHORS' CONTRIBUTION

MB – Acquisition and analysis of clinical data; writing draft

MM – Data management; critical revision of manuscript

MP – Data management; statistical analysis

MD – Data management; analysis of susceptibility profiles; writing draft

LA – Acquisition and analysis of clinical data; writing draft

LI – Acquisition and analysis of clinical data; writing draft

RS – Data management; analysis of susceptibility profiles; writing draft

CONFLICT OF INTEREST

None to declare.

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