Clin. Lab. 2023;69:XXX-XXX

ORIGINAL ARTICLE

Trends of Antibiotic Resistance in Multidrug-Resistant Pathogens Isolated from Blood Cultures in a Four-Year Period

Lutfiye Oksuz, Goknur Altay, Zerrin Aktas

Department of Medical Microbiology, Istanbul Faculty of Medicine, Istanbul, Turkey

SUMMARY

Background: **Multidrug-resistant organisms cause serious infections with significant morbidity and mortality in the worldwide. These organisms have been identified as urgent and serious threats by CDC. The aim of this study was to determine the prevalence and changes of antibiotic resistance of multidrug-resistant pathogens isolated from blood cultures over a four-year period in a tertiary-care hospital.**

Methods: **Blood cultures were incubated in a blood culture system. Positive signalling blood cultures were subcultured on 5% sheep-blood agar. Identification of isolated bacteria was performed using conventional or automated identification systems. Antibiotic susceptibility tests were performed by disc diffusion and/or gradient test methods, if necessary, by automated systems. The CLSI guidelines were used for interpretation of antibiotic susceptibility testing of bacteria.**

Results: **The most frequently isolated Gram-negative bacteria was *Escherichia coli* (33.4%) followed by *Klebsiella pneumoniae* (21.5%). ESBL positivity was 47% for *E. coli*, 66% for *K. pneumoniae*. Among *E. coli*, *K. pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* isolates, carbapenem resistance was 4%, 41%, 37%, and 62%, respectively. Carbapenem resistance of *K. pneumoniae* isolates has increased from 25% to 57% over the years, and the highest rate (57%) occured during the pandemic period. It is noteworthy that the aminoglycoside resistance in *E. coli* isolates gradually increased from 2017 to 2021. The rate of methicillin-resistant *S. aureus* (MRSA) was found to be 35.5%.**

Conclusions: **Increased carbapenem resistance in *K. pneumoniae* and *A. baumannii* isolates is noteworthy, but carbapenem resistance in *P. aeruginosa* decreased. It is of great importance for each hospital to monitor the increase in resistance in clinically important bacteria, especially isolated from invasive samples, in order to take the necessary precautions in a timely manner. Future studies involving clinical data of patients and bacterial resistance genes are warranted.**

(Clin. Lab. 2023;69:xx-xx. DOI: 10.7754/Clin.Lab.2023.230311)

Correspondence:
Lutfiye Oksuz
Istanbul University
Istanbul Faculty of Medicine
Department of Medical Microbiology
34093 Capa-Fatih/Istanbul
Turkey
Email: oksuzl@istanbul.edu.tr
ORCID: 0000-0002-6722-141X

Manuscript accepted March 14, 2023

KEYWORDS

Blood culture, multidrug-resistance, extended-spectrum beta lactamase, carbapenem resistance, surveillance

INTRODUCTION

Sepsis is a major public health problem [1,2]. In 2017, the World Health Assembly (WHA) and the World Health Organization (WHO) declared that reducing the global burden of sepsis was a priority [2]. ESKAPEEc pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*,

Pseudomonas aeruginosa, *Enterobacter* spp. and *Escherichia coli*) largely cause serious infections such as bloodstream infections and constitute an important part of multidrug-resistant organisms [3]. Extended-spectrum β-lactamase-producing *Enterobacterales* (ESBL-E), carbapenem-resistant *Enterobacterales* (CRE), and difficult-to-treat resistant *Pseudomonas aeruginosa* (DTR) isolates are multidrug-resistant organisms and cause a wide variety of infections with significant morbidity and mortality. These organisms have been identified as urgent and serious threats by the Centers for Disease Control and Prevention (CDC) [4]. WHO published a list of pathogens in urgent need of antibiotic development in 2017, carbapenem-resistant *A. baumannii*, *Enterobacterales*, *P. aeruginosa*; vancomycin-resistant *E. faecium* and methicillin/vancomycin-resistant *S. aureus* were the priority groups [5].

Antimicrobial resistant pathogens caused more than 2.8 million infections and more than 35,000 deaths annually from 2012 to 2017 according to the 2019 CDC Report on Antibiotic Resistance Threats in USA [4,6]. It is estimated that by 2050, approximately 10 million deaths will occur annually from drug-resistant microorganisms [7]. While cephalosporin and carbapenem class antibiotics are widely used in the treatment of serious infections caused by *Enterobacterales*, these options are no longer preferred with the acquisition of genes encoding ESBL and carbapenemase enzymes [8]. Serious infections caused by isolates of CRE have often been associated with high mortality rates exceeding 40% [9].

Empirical treatment decisions should be guided by the most likely pathogens, the severity of disease, the potential source of infection, and additional patient-specific factors [6]. It is important to monitor multidrug-resistant pathogens because they cause high morbidity, mortality, and economic losses [10,11]. Many countries are trying to establish and develop antimicrobial resistance (AMR) surveillance networks [12]. AMR surveillance in our country has been followed by the National Antimicrobial Resistance Surveillance System (NARSS) since 2011. Also, our hospital data is submitted to the Central Asian and Eastern European Antimicrobial Resistance Surveillance Network (CAESAR).

The aim of this study was to determine the prevalence and antibiotic resistance of multidrug-resistant pathogens (*E. coli*, K. *pneumoniae*, *P. aeruginosa*, *A. baumannii*, *S. aureus*, *E. faecium*/*E. faecalis*, *Steptococcus pneumoniae*) isolated from blood cultures over a four-year period.

MATERIALS AND METHODS

Study Design

We conducted a retrospective cohort study in a university hospital during a four-year period (January 2017 - December 2020). Our hospital is a 1,300 bed tertiary care tertiary care hospital in Istanbul, Turkiye. In this study, antibiotic susceptibility of multidrug-resistant bacteria isolated from blood cultures during the four-year period were evaluated. The first isolate from each patient was included in the study. Demographic information of patients was obtained from the Hospital Information Management System.

Microbiological procedures

Microbiological data including patient age, gender, outpatient/inpatient status, clinics, isolated bacteria, and antimicrobial susceptibility patterns were obtained from the laboratory information system (LIS). Blood cultures sent from different hospital wards were inoculated into blood culture bottles and incubated for five days in a blood culture instrument (Becton Dickinson, Sparks, MD, USA). Positive signaling blood cultures were processed for Gram staining and subcultured on 5% sheep blood agar. Identification of bacteria isolated from positive blood culture bottles was performed using conventional methods, if necessary, by automated identification systems (Phoenix 100, Beckton Dickinson, USA; VITEK2, bioMeriueux, France). Antibiotic susceptibility tests were performed by the Kirby Bauer disc diffusion method and gradient test method (E test, bioMerieux, France), if necessary, by automated systems (Phoenix, USA; VITEK, bioMerieux, France), then interpreted according to the recommendations of the CLSI guidelines. Screening test and double-disc synergy test were used to detect extended-spectrum beta-lactamase producing isolates [13]. Multidrug-resistance (MDR) is defined as non susceptibility to at least one agent in three antibiotic classes, and pandrug-resistance (PDR) is defined as non susceptibility to all agents [14].

Data analysis

Microsoft Excel 2016 software (Microsoft Corp., USA) was used to analyze the data by years, to determine bacterial numbers and resistance rates and to prepare of the graphs. Isolates intermediate to antibiotics were accepted as resistant.

Statistical analysis

The Statistical Package for Social Science for Windows (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA) 26.0 package program was used for the evaluation of the results. Age ranges, mean, and standard deviation values of the patients were determined using SPSS program. The chi-squared test was used for the significance of the difference between categorical variables, the ANOVA test for the significance of the difference between the means of more than two independent groups, and the paired samples *t*-test for the significance of the difference between the means of paired-related groups. The statistical significance was defined as a p-value of < 0.05.

Table 1. Demographic data of the patients by years (n).

Demographic data		2017	2018	2019	2020
Patients					
Pediatric		**74**	**26**	**67**	**63**
Adult		**156**	**86**	**249**	**239**
Gender	**Female**	**112**	**52**	**169**	**136**
	Male	**118**	**60**	**147**	**166**
Clinic					
Outpatient		**66**	**22**	**113**	**116**
Inpatient		**129**	**84**	**188**	**145**
Intensive care unit		**35**	**6**	**15**	**41**
Hospital ward					
Internal medicine		**134**	**81**	**227**	**116**
Pediatric clinics		**61**	**21**	**54**	**51**
Surgery clinics		**35**	**10**	**35**	**62**
Pandemic clinic		**-**	**-**	**-**	**73**

Table 2. The number of bacteria isolated from blood cultures by years (n).

Bacteria	2017	2018	2019	2020	Total
Escherichia coli	**76**	**39**	**113**	**99**	**327**
Klebsiella pneumoniae	**46**	**14**	**62**	**80**	**202**
Pseudomonas aeruginosa	**14**	**11**	**13**	**13**	**51**
Acinetobacter baumannii	**6**	**2**	**7**	**14**	**29**
Staphylococcus aureus	**61**	**32**	**84**	**67**	**244**
Enterococcus faecium	**12**	**4**	**13**	**14**	**43**
Enterococcus faecalis	**11**	**6**	**17**	**12**	**46**
Streptococcus pneumoniae	**4**	**4**	**7**	**3**	**18**
Total	**230**	**112**	**316**	**302**	**960**

RESULTS

This monocentric, retrospective, observational study evaluated the ESKAPEEc pathogen from 960 patients isolated from blood cultures from January 2017 to December 2020. The average age of the pediatric patients was 7.36 ± 4.3, while the average age of the adult was 63.7 ± 18.2. No statistically significant difference was found between male and female patients ($p = 0.260$). A statistically significant difference was found between the number of pediatric and adult patients ($p = 0.002$). A statistically significant difference was found between the number of outpatients and inpatients ($p = 0.001$). There was a significant difference between the patients from internal medicine (n = 558) and those from pediatrics (n = 187), surgery (n = 142), and pandemic (n = 73) clinics ($p < 0.05$).

Demographic data of the patients by years were given in Table 1. The numbers of bacteria isolated by years were shown in Table 2. The mean ESBL rates in *E. coli* and *K. pneumoniae* isolates was found to be 47% and 66%, respectively, in the four-year period (Figure 1). Figure 2 indicates the ESBL rates in *E. coli* and *K. pneumoniae* isolates according to the status of patients in outpatient, inpatient, and intensive care units. Figure 3 shows the rates of resistance in Gram-negative bacteria to antibiotics including third generation cephalosporins, carbapenems, aminoglycosides, fluoroquinolones. Carbapenem resistance was not detected in *E. coli* isolates in 2017 and 2018, and the rate was below 10% in 2019 and

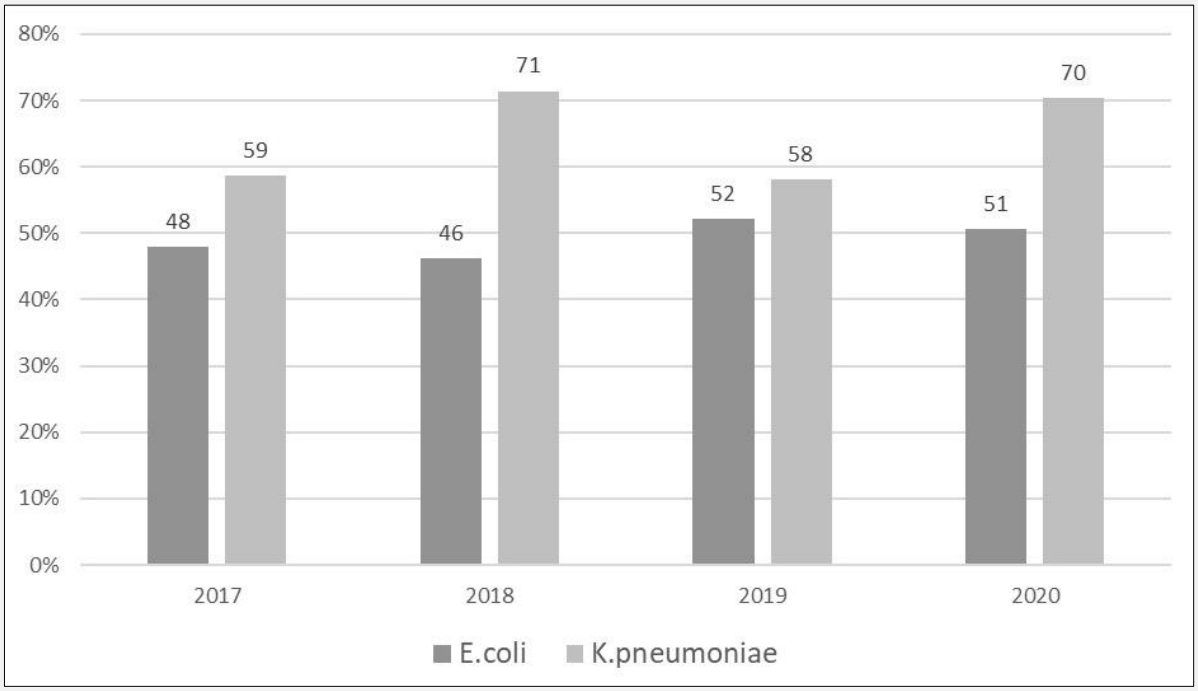

Figure 1. ESBL rates in *E. coli* and *K. pneumoniae* isolates by years (%).

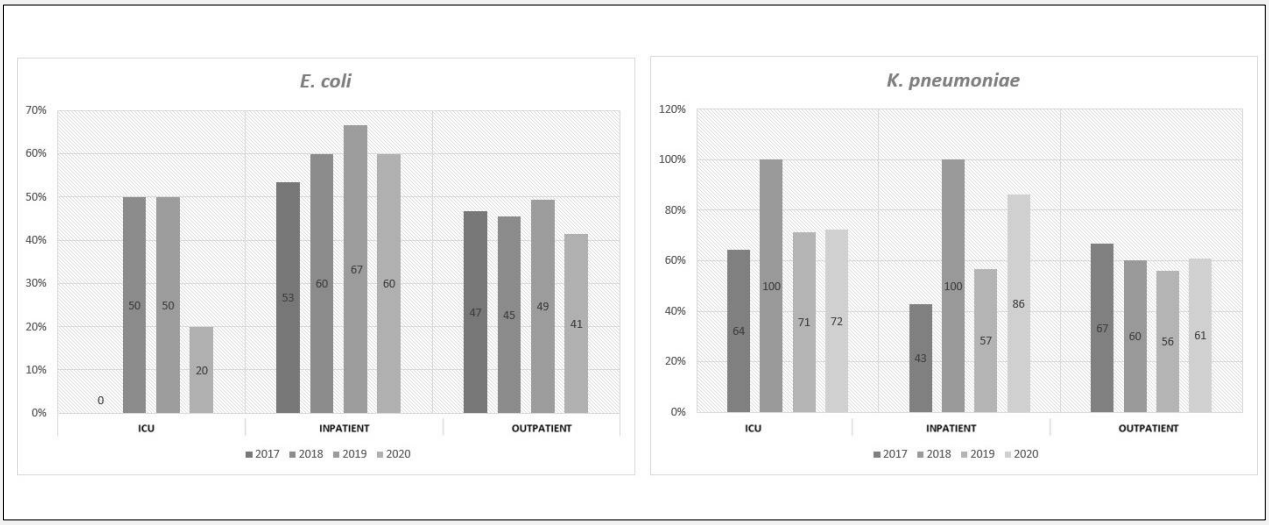

Figure 2. ESBL rates in *E. coli* and *K. pneumoniae* isolates according to the status of patients in outpatient, inpatient, and intensive care units (ICU) (%).

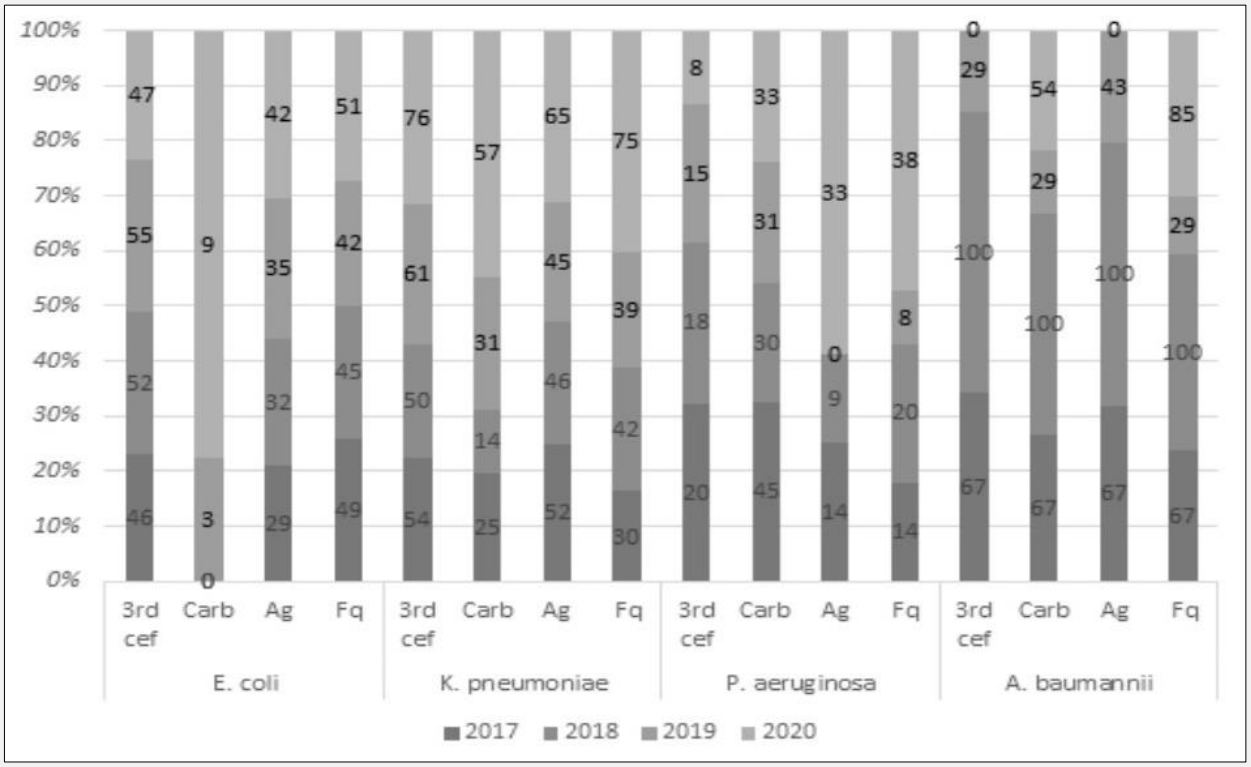

Figure 3. The rates of resistance to antibiotics in Gram-negative bacteria by years (%).

2020. Carbapenem resistance in *K. pneumoniae* isolates increased gradually over time, but decreased gradually in *P. aeruginosa* isolates. Carbapenem resistance rates exceeding 50% in *A. baumanni* isolates are noteworthy (Figure 4). Figure 5 shows the differences of MRSA rates according to the status of patients in outpatient, inpatient, and ICU.

DISCUSSION

In this study, the serious pathogens (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*, *S. aureus*, *E. faecium*/*E. faecalis*, *S. pneumoniae*) isolated from blood cultures sent to the laboratory from January 2017 to October 2022 and their antibiotic resistance were analyzed retrospectively, based on the isolates sent to NARSS. Our surveillance data showed that *E. coli* (34%), *S. aureus* (25%), and *K. pneumoniae* (21%) were the most common pathogens in our hospital from 2017 to 2021 (Table 2). Similar rates were reported from Italy and China [15,16].

Antibiotic resistance in fermentative Gram-negative bacteria

Although there was no significant difference in the antibiotic resistance rates of *E. coli* isolates by years (p = 0.663), a significant difference was found in term of resistance change in *K. pneumoniae* isolates (p = 0.016). Resistance to third-generation cephalosporins was 53.4% in *E. coli* isolates from Turkiye according to the 2020 data of ECDC, and 76.9% in *K. pneumoniae* isolates [17]. ESBL rates in *E. coli* and *K. pneumoniae* isolates in our study were shown in Figure 1. Although ESBL rates in *E. coli* isolates do not change much over the years, there was an increase (59% - 75%) in ESBL rates of *K. pneumoniae* isolates. ESBL rates in *K. pneumoniae* isolates in inpatient and intensive care unit (ICU) patients were similar, and lower rates were found in outpatients. In a Suzuk's study conducted on 509 isolates from 26 hospitals within the scope of NARSS in 2019, the rate of ESBL positivity was 59.1% in *E. coli* and 75.4% in *K. pneumoniae* isolates [18]. Our findings were lower compared with both studies of ECDC and Suzuk et al. Resistance of *K. pneumoniae* to 3rd generation cephalosporins had a statistically significant difference by years (p = 0.009). Also, there is a statistically

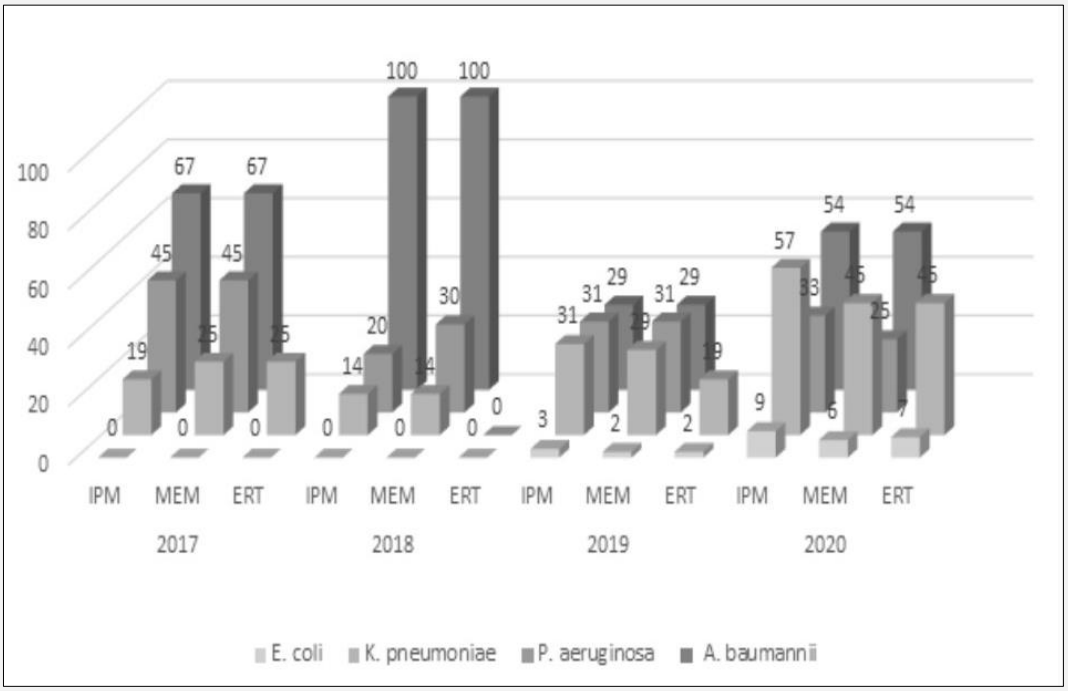

Figure 4. Trends of carbapenem resistance in Gram-negative bacteria in a four-year period (%).

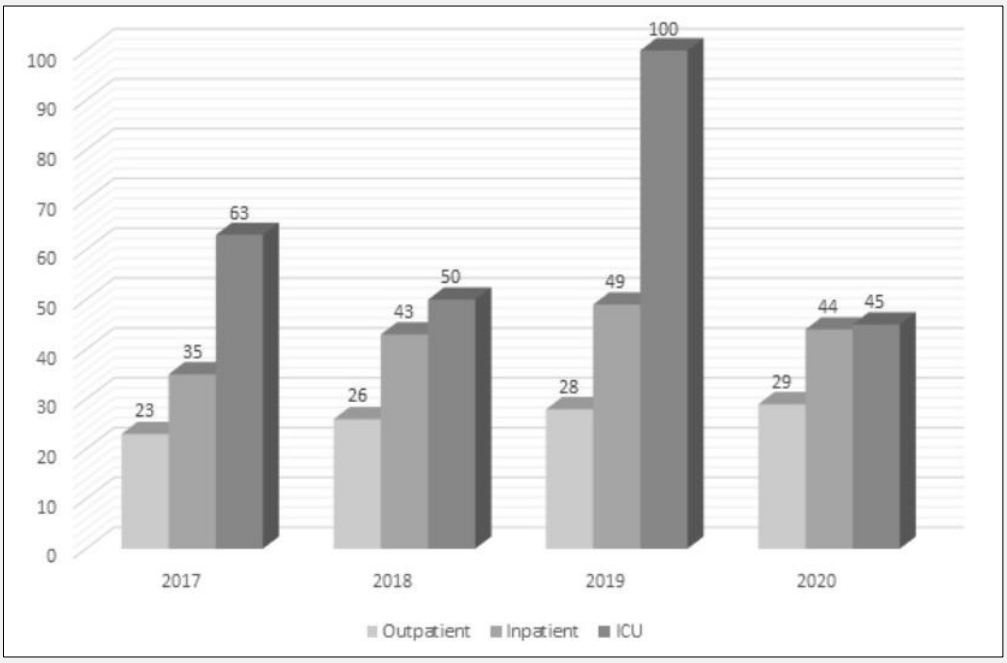

Figure 5. The rates of MRSA according to the status of patients in outpatient, inpatient, and intensive care units (ICU) (%).

significant difference between ESBL rates in *E. coli* isolates and in *K. pneumoniae* isolates (p = 0.004). In addition, ESBL positivity in Gram-negative bacteria has a statistically significant difference over the years (p = 0.015). In a study from Italy on ESCAPEEc pathogens and antibiotic resistance, increased rates of ESBL positive *E. coli* were reported over a nine-year period [15]. The incidence of ESBL-E in the USA increased by 53% from 2012 to 2017 [19]. In our study, similarly, the rate of ESBL-positive *K. pneumoniae* isolates has increased over the years, especially during the pandemic period. In contrast, in a recent study which is reported from Italy, MDR *Enterobacterales* isoates were low, which differs from our study. The authors reported that the low MDR rate is due to the rational use of antibiotics during the pandemic period and continuous hand hygiene training to healthcare personnel [20].

Carbapenems are recommended as primary therapy of ESBL-E infections [21]. However, the increasing use of carbapenems in the clinical settings has created a selective pressure for the emergence of carbapenem resistance [9]. The CDC defines CRE as members of the order *Enterobacterales* that are resistant to at least one carbapenem antibiotic or produce a carbapenemase enzyme [22]. In the study by Suzuk et al, carbapenem resistance was reported to be 17% - 25% in *E. coli* and 48% - 58% in *K. pneumoniae* isolates [18]. According to the 2020 data of ECDC, among *K. pneumoniae* isolates from Turkiye, the rates of carbapenem resistance were reported as 48.2% and 3.7% in *E. coli* isolates [17]. Our results (mean 4.5%; min. 0% - max. 9%) for *E. coli* isolates were lower than the results of Suzuk et al. and slightly higher than the results of ECDC. For *K. pneumoniae* isolates, these rates were slightly lower 36% (14 - 57%) than in both studies (Figure 4). Similar results were reported from Thailand [23]. Compared to the rates in this study and in our country, rather low (< 0.5% for *E. coli*, 1 - 10% for *K. pneumoniae*) carbapenem resistance rates were reported from China [16]. However, carbapenem resistance rates of up to 54% have been reported in *K. pneumoniae* isolates in most countries [24]. For example, a significant increase (from 4.2 to 51.6%) in carbapenemase-producing *K. pneumoniae* were reported from Italy over a nine-year period [15]. CRE isolates are responsible for more than 13,000 nosocomial infections and contribute to more than 1,000 deaths per year in the United States [4]. Carbapenem-resistant *K. pneumoniae* (CRKP) strains are the most clinically significant CRE isolates. *K. peumoniae* carbapenemase (KPC) was first reported in the USA in 2001 and has been spreading ever since. Between 2005 and 2010, an increase in CRKP isolates causing invasive infections was reported across Europe [9]. According to the data of an online tool showing the current resistance rates and antibiotic use in various countries on an interactive map, carbapenem resistance rates in *K. pneumoniae* in Turkiye increased from 15% to 45% from 2013 to 2019 [25]. Similarly, imipenem resistance of *K. pneumoniae* isolates in our study has increased from 19% to 57% over the years, and the highest rate (57%) was observed during the pandemic period (Figure 4). In addition, resistance to carbapenems was found statistically significantly different in Gram-negative bacteria over the years (p = 0.000). During the pandemic period which had significant effects all over the world, an increase was observed in serious pathogens (e.g., *K. pneumoniae*) isolated from blood cultures [26, 27]. In this study, *K. pneumoniae*, *E. coli*, and *S. aureus* were prominent among the bacteria isolated from blood cultures in the first year of the pandemic (Table 2).

Resistance to aminoglycosides in *E. coli* and *K. pneumoniae* isolates from Turkiye was 23.7% and 46.6%, respectively, according to the 2020 data of ECDC [17]. In the present study, a statistically significant difference was found in the resistance of Gram-negative bacteria to aminoglycosides over the years (p = 0.007). We found that the rates of aminoglycosides were 34.5% (29 - 42%) and 52% (45 - 65%) in *E. coli* and *K. pneumoniae*, respectively (Figure 3). Our findings for *E. coli* and *K. pneumoniae* were higher than those of ECDC. The prevalence of resistance to aminoglycosides appears to be on the rise in our hospital. It is noteworthy that the aminoglycoside resistance in *E. coli* isolates increased from 28% to 40% for gentamicin, from 29% to 41% for tobramycin, and from 1% to 5% for amikacin from 2017 to 2020. However, there was a decrease in resistance to gentamicin and tobramycin, and an increase in resistance to amikacin in *K. pneumoniae* isolates. The difference from ECDC data may be due to changes in antibiotic use policies between regional and countries. Since ESBL enzymes and genes for resistance to aminoglycosides, fluoroquinolones, etc. are carried on the same plasmids, increasing resistance to aminoglycosides are in line with the increase in our ESBL rates, except amikacin. Similar results for *E. coli* isolates were reported in a multicenter from our country and from China [16,28] which was different (72% for aminoglycosides) from that in Iran [29] and Egypt (57 - 72%, for aminoglycosides) [30].

Resistance to fluoroquinolones in *E. coli* and *K. pneumoniae* isolates from Turkiye was found to be 50.1% and 69%, respectively, according to ECDC [17]. In the present study, a statistically significant difference was found in the resistance of Gram-negative bacteria to fluoroquinolones over the years (p = 0.000). We found that fluoroquinolon resistance in *E. coli* and *K. pneumoniae* isolates was 47% (42 - 51%) and 46.5% (30 - 75%), respectively. Our results for *E. coli* isolates are consistent with data from ECDC, but it was lower for *K. pneumoniae* (Figure 3). This may be because our study included data from a single center. Fluoroquinolone resistance in *E. coli* isolates was consistent with the work of Tian et al., but differed for *K. pneumoniae* isolates, with lower rates of resistance reported (51% vs. 25%) [16].

Antibiotic resistance in nonfermentative Gram-negative bacteria

Multidrug-resistant (MDR) *P. aeruginosa* is defined as *P. aeruginosa* not susceptible to at least one antibiotic from at least three classes of antibiotics commonly used for *P. aeruginosa* [31]. The definition of "difficult to treat resistance - DTR" has been used for these bacteria since 2018 [32]. DTR-*P. aeruginosa* usually develops as a result of the interaction of multiple complex resistance mechanisms. These mechanisms include decreased expression of outer membrane porins (*Opr*D), hyper production of AmpC enzymes, upregulation of efflux pumps, and mutations in penicillin-binding protein targets [33,34]. Chromosomal resistance leading to decreased susceptibility to most β-lactams, aminoglycosides, tetracyclines, and carbapenems in *P. aeruginosa* is mediated through overexpression of AmpC cephalosporinases, efflux pumps, and loss of outer membrane porins. *P. aeruginosa* may also acquire resistance genes encoding ESBLs and carbapenemases through horizontal gene transfer. As a result, MDR and PDR isolates are increasing in the clinic and options for treating infections caused by these isolates remain limited [35]. The rates of MDR *P. aeruginosa* isolates were 14%, 9%, 15%, and 38% from 2017 to 2020 in our study. PDR *P. aeruginosa* isolates were found to be 14% in 2017 and 9% in 2018, respectively, but PDR isolates were not detected in 2019 and 2020.

There was a statistically significiant difference in the antibiotic resistance rates of *P. aeruginosa* isolates by years (p = 0.001). Ceftazidime resistance in *P. aeruginosa* isolates was 15% (8 - 21%), and it has a statistically significant difference by years (p = 0.009). In a study conducted in our country, ceftazidime resistance in *P. aeruginosa* isolates from blood cultures was reported as 43%, higher than our result [36]. In a study from China covering the years 2008 - 2011, ceftazidime resistance in *P. aeruginosa* isolates was found to be 6% in the first two years, while it increased to 33% and 37% in the last two years [37]. In another study conducted in China the resistance to ceftazidime in *P. aeruginosa* isolates was reported that as 6.9% [16]. In the GLASS study conducted in Thailand, resistance to ceftazidime in *P. aeruginosa* isolates was reported to be 9% in community-acquired infections and 33% in hospital-acquired infections [23]. The resistance to carbapenems in *P. aeruginosa* isolates in Turkey were reported as 36.2% in ECDC data [17]. In the present study, the rate of carbapenem resistance in *P. aeruginosa* isolates was 32% (20 - 45%). Fortunately, carbapenem resistance has decreased gradually from 45% to 33% from 2017 to 2020 (Figure 3). The decrease may be due to hospital infection control measurements taken for patients in our hospital. Similarly, the decrease from 55% to 44% in carbapenem resistance rates were also found in Italy in a nine-year period [15]. Patients with infections caused by MDR/XDR/PDR pathogens are at high risk of receiving inadequate initial antimicrobial therapy [14]. It has been reported that the combination of ceftazidime-colistin provides increased activity against ceftazidime-resistant and MDR *P. aeruginosa* [38]. Infectious Diseases Society of America (IDSA) recommends ceftolozane-tazobactam, ceftazidime-avibactam or imipenem-relebactam for the treatment of DTR *P. aeruginosa* infections outside the urinary tract [6]. In our study, the rates of resistance to aminoglycosides in *P. aeruginosa* isolates were 14% (0 - 33%). Fluoroquinolone resistance in *P. aeruginosa* isolates was found as 31% in the ECDC study. Our findings were lower than those with the rate of 20% (8 - 38%) in our study. One study reported that resistance to ciprofloxacin in *P. aeruginosa* was associated with its ability to form biofilm [10]. In a study conducted in Italy over a nine-year period, a decrease (from 46% to 36%) was detected in ciprofloxacin resistance of *P. aeruginosa* isolates from blood cultures [15]. Antibiotic resistance trends in *P. aeruginosa* isolates of our study by years are shown in Figure 3.

A statistically significiant difference was found in the antibiotic resistance rates of *A. baumannii* isolates by years (p = 0.000). Ceftazidime resistance in *A. baumannii* isolates was 49% (29 - 100) in our study. Carbapenem-resistant *A. baumanni* is at the top of the WHO's list of pathogens that pose a threat to human health [5]. Carbapenem and fluoroquinolone resistance rates in *A. baumannii* isolates by ECDC were 93.1% and 93.6%, respectively [15]. In this study, carbapenem and fluoroquinolone resistance rates were 62.5% (29 - 100%) and 70.25% (29 - 100%) in *A. baumannii* isolates, respectively. These rates were slightly lower compared with the ECDC study. Also, the resistance of *A. baumannii* to carbapenems has a statistically significant difference by years (p = 0.000). Similarly, in a study from Hungary investigating the increase in resistance in ESKAPE pathogens over a 10-year period, carbapenem-resistant *A. baumannii* was reported as the pathogen with the highest resistance increase over the years [11]. In the GLASS study conducted in Thailand, it has been reported that meropenem resistance of *A. baumannii* isolates was 30% in community-acquired bacteremia patients, but 75% in hospital-acquired bacteremia [23]. Although we could not differentiate between community and hospital-acquired bacteremia in our study, our results were consistent with these data. In the present study, the rates of resistance to aminoglycosides *A. baumannii* isolates were 56% (43 - 100%) by years. Resistance of *P. aeruginosa* and *A. baumannii* isolates to aminoglycosides has a statistically significant difference (p = 0.006). The high resistance rates to antibiotic classes of *A. baumanni* isolates are remarkable, because the resistance rates to carbapenems (62.5%), aminoglycosides (56%), and fluoroquinolones (70.2%) are above 50%. These numbers indicate the high MDR rate in *A. baumannii* isolates. Similarly, the high rates of resistance (> 50%) for *A. baumannii* were reported from Spain [39]. Also, a study from Italy noting the increase in MDR *A. baumnanii* isolates is consistent with our data [20]. The high rates of resistance might be related

to several factors including predisposing factors, previous antibiotic use, long-term hospitalization, intensive care unit stay, etc. As a result, treatment options are limited in infections caused by *A. baumannii*, and the antibiotic combinations should be preferred.

Antibiotic resistance in Gram-positive bacteria

From 2017 to 2020, 244 *S. aureus* was isolated from blood cultures, of which 90 were methicillin-resistant. In the present study, resistance to antibiotics in Gram-positive bacteria has a statistically significant difference over years (p = 0.000). The median rate of MRSA was found as 35.5% (31% - 40%) in a four-year period. Figure 5 shows the rates of MRSA according to the status of patients in outpatient, inpatient, and ICU. In a study from Italy on ESCAPEEc pathogens and antibiotic resistance, an increase (from 34% to 44%) in MRSA rates was reported over a nine-year period [15]. Our result was similar compared to the 2020 data of ECDC [17]. However, there were only minor increases in erythromycin resistance (from 38% to 41%) and clindamycin resistance (from 25% to 27%) of *S. aureus* isolates in our study. Tian et al. reported erythromycin and clindamycin resistance in *S. aureus* isolates as 61% and 40%, respectively, and they did not detect resistance to vancomycin, teicoplanin, and linezolid [16]. Similarly, resistance to glycopeptides and linezolid were not detected among *S. aureus* isolates in present study.

In the present study, a significant difference was found in the resistance change in *E. faecalis* isolates (p = 0.023). In contrast, Orosz et al. reported that *E. faecium* isolates show a steadily increasing resistance and may result in 86% resistance for vancomycin-resistant *enterococcus* (VRE) by 2030 [11]. According to the 2020 data of ECDC, the rate of vancomycin-resistant (VR-) *E. faecium* was 15.4% [17]. In our study, the rate of VR-*E. faecium* was higher than this data as 12.25% (0 - 50%), while it was found as low as 1.5% (0 - 14%) in VR-*E. faecalis* isolates. The higher (> 21%) rates compared to our results for *E. faecium* were found in Spain [39]. The rate of resistance to glycopeptides in the study of Tian et al. was consistent with our study (0.9%) for *E. faecalis*, but they found very low (1.5 - 2.6%) resistance rates for *E. faecium* compared to our rates [16]. Although routine VRE screenings are conducted by the hospital control committee in our hospital, high resistance rates are a concern.

The number of *S. pneumoniae* isolates isolated from blood cultures during the four-year period is quite low. This may be because pneumococcal conjugated vaccines are included in the national childhood immunization calendar and have been routinely administered since 2007. Resistance to penicillin and cefotaxime was not detected in *S. pneumoniae* isolates. Unlike the results of this study, in a study conducted in China, it was reported that there was 3.9% and 5.1% resistance to penicillin and cefotaxime, respectively, in *S. pneumoniae* isolates from blood cultures [16].

Limitations of the study

The study has some limitations that do not significantly affect the results. One of these limitations is that it does not include all bacteria isolated from blood cultures as it is based on NARSS data. Another limitation is that some bacteria were isolated in small numbers in certain years (e.g., *A. baumannii*, *S. pneumoniae*, *Enterococcus* spp). Additionally, colistin resistance in Gram-negative bacteria could not be discussed, since the guidelines recommended colistin susceptibility to be performed by broth microdilution method and could not be routinely tested for each isolate. In addition, we could not investigate resistance genes because it was a retrospective study. Our findings will shed light on similar studies to be conducted in our country or neighboring countries in the future and will guide the treatment of patients infected with multidrug-resistant bacteria.

CONCLUSION

Our surveillance data showed that MDR isolates such as ESBL positive *E. coli*, ESBL positive *K. pneumoniae* and MRSA are still a serious concern. It is noteworthy that carbapenem resistance in *K. pneumoniae* and *A. baumannii* isolates increased over time. Fortunately, the rate of resistance to carbapenems in *P. aeruginosa* isolates gradually decreased over a four-year period. It is of great importance for each hospital to monitor the increase in resistance in clinically important bacteria, especially isolated from invasive samples, in order to take the necessary precautions in a timely manner. Future studies involving clinical data of patients and bacterial resistance genes are warranted.

Acknowledgment:

We would like to thank to Prof. Haluk Zulfikar, Director of the Center, Istanbul University Rectorate, Statistics Application and Research Center, and his assistant Esra Kucukbas, for contribution on statistical analyses. We thank all laboratory staff and medical microbiology specialists for their contribution to the examination of clinical specimens.

Source of Funds:

No specific grants were received for this study from funding agencies in the public, commercial, or not-for-profit sectors.

Ethics Approval:

Ethics approval was not received due to the retrospective nature of the study.

Consent for Publication:
The manuscript is not under publication or consideration for publication elsewhere. If accepted, it is allowed to be published in the journal.

Declaration of Interest:
The authors declare that they have no conflicts of interest.

References:

1. Rhee C, Dantes R, Epstein L, et al. Incidence and Trends of Sepsis in US Hospitals Using Clinical vs Claims Data, 2009-2014. JAMA 2017;318:1241-9. (PMID: 28903154)

2. Kempker JA, Wang HE, Martin GS. Sepsis is a preventable public health problem. Crit Care 2018;22:116. (PMID: 29729670)

3. De Socio GV, Rubbioni P, Botta D, et al. Measurement and prediction of antimicrobial resistance in bloodstream infections by ESKAPE pathogens and Escherichia coli. J Glob Antimicrob Resist 2019;19:154-60. (PMID: 31112804)

4. Center for Disease Control and Prevention (CDC)(2019). Antibiotic Resistance Threats In The United States. PDF. Atlanta: U.S. Department of Health and Human Services. https://www.cdc.gov/drugresistance/biggest-threats.html#:~:text=More%20than%202.8%20million%20antimicrobial,people%20die%20as%20a%20result

5. Willyard C. The drug-resistant bacteria that pose the greatest health threats. Nature 2017;543:15. (PMID: 28252092)

6. Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. Infectious Diseases Society of America 2022 Guidance on the Treatment of Extended-Spectrum beta-lactamase Producing Enterobacterales (ESBL-E), Carbapenem-Resistant Enterobacterales (CRE), and Pseudomonas aeruginosa with Difficult-to-Treat Resistance (DTR-P. aeruginosa). Clin Infect Dis 2022;75:187-212. (PMID: 35439291)

7. de Kraker ME, Stewardson AJ, Harbarth S. Will 10 Million People Die a Year due to Antimicrobial Resistance by 2050? PLoS Med 2016;13:e1002184. (PMID: 27898664)

8. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 2005;18:657-86. (PMID: 16223952)

9. De Oliveira DMP, Forde BM, Kidd TJ, et al. Antimicrobial Resistance in ESKAPE Pathogens. Clin Microbiol Rev 2020; 33(3):e00181-19. (PMID: 32404435)

10. Cepas V, Lopez Y, Munoz E, et al. Relationship Between Biofilm Formation and Antimicrobial Resistance in Gram-Negative Bacteria. Microb Drug Resist 2019;25:72-9. (PMID: 30142035)

11. Orosz L, Lengyel G, Anosi N, Lakatos L, Burian K. Changes in resistance pattern of ESKAPE pathogens between 2010 and 2020 in the clinical center of University of Szeged, Hungary. Acta Microbiol Immunol Hung 2022;69:27-34. (PMID: 35084364)

12. Gandra S, Alvarez-Uria G, Turner P, Joshi J, Limmathurotsakul D, van Doorn HR. Antimicrobial Resistance Surveillance in Low- and Middle-Income Countries: Progress and Challenges in Eight South Asian and Southeast Asian Countries. Clin Microbiol Rev 2020;33:e00181-19. (PMID: 32522747)

13. Clinical and Laboratory Standards Institute (CLSI)(2020). Performance standards for antimicrobial susceptibility testing CLSI Supplement M100. 30th ed. USA: Wayne Pa. Available from: www.clsi.org. Accessed at November 12, 2022.

14. Horcajada JP, Montero M, Oliver A, et al. Epidemiology and Treatment of Multidrug-Resistant and Extensively Drug-Resistant Pseudomonas aeruginosa Infections. Clin Microbiol Rev 2019;32: e00031-19. (PMID: 31462403)

15. De Angelis G, Fiori B, Menchinelli G, et al. Incidence and antimicrobial resistance trends in bloodstream infections caused by ESKAPE and Escherichia coli at a large teaching hospital in Rome, a 9-year analysis (2007-2015). Eur J Clin Microbiol Infect Dis 2018;37:1627-36. (PMID: 29948360)

16. Tian L, Sun Z, Zhang Z. Antimicrobial resistance of pathogens causing nosocomial bloodstream infection in Hubei Province, China, from 2014 to 2016: a multicenter retrospective study. BMC Public Health 2018;18:1121. (PMID: 30219056)

17. European Centre for Disease Prevention and Control & World Health Organization. Regional Office for Europe. (2022). Antimicrobial resistance surveillance in Europe 2022 - 2020 data. World Health Organization. Regional Office for Europe. Available at https://apps.who.int/iris/handle/10665/351141. Accessed at November 12, 2022.

18. Suzuk Yildiz S, Simsek H, Bakkaloglu Z, et al. [The Epidemiology of Carbapenemases in Escherichia coli and Klebsiella pneumoniae Isolated in 2019 in Turkey]. Mikrobiyol Bul 2021;55:1-16. (PMID: 33590977)

19. Jernigan JA, Hatfield KM, Wolford H, et al. Multidrug-Resistant Bacterial Infections in U.S. Hospitalized Patients, 2012-2017. N Engl J Med 2020;382:1309-19. (PMID: 32242356)

20. Cuntro M, Manisco A, Guarneri D, et al. Blood stream infections during the first wave of COVID-19. A short microbiological retrospective picture at Papa Giovanni XXIII Hospital, Bergamo, Italy. New Microbiol 2021;44:51-8. (PMID: 33755185)

21. Harris PNA, Tambyah PA, Lye DC, et al. Effect of Piperacillin-Tazobactam vs Meropenem on 30-Day Mortality for Patients With E coli or Klebsiella pneumoniae Bloodstream Infection and Ceftriaxone Resistance: A Randomized Clinical Trial. JAMA 2018;320:984-94. (PMID: 30208454)

22. Centre for Disease Prevention and Control (CDC)(2015). Facility Guidance for Control of Carbapenem-resistant Enterobacteriaceae (CRE). PDF. 2015-Update. https://www.cdc.gov/hai/pdfs/cre/CRE-guidance-508.pdf Accessed at November 12, 2022.

23. Sirijatuphat R, Sripanidkulchai K, Boonyasiri A, et al. Implementation of global antimicrobial resistance surveillance system (GLASS) in patients with bacteremia. PLoS One 2018;13: e0190132. (PMID: 29298323)

24. World Health Organization (2014). Antimicrobial resistance: global report on surveillance. PDF. Available at: https://apps.who.int/iris/handle/10665/112642. Accessed at November 12, 2022.

25. ResistanceMap: Antibiotic resistance: OneHealthTrust (2022). Available from: https://resistancemap.onehealthtrust.org/AntibioticResistance.php Accessed on November 12, 2022.

26. Brink AJ, Richards G, Tootla H, Prentice E. Epidemiology of Gram-negative bacteria during coronavirus disease 2019. What is the real pandemic? Curr Opin Infect Dis 2022;35:595-604. (PMID: 36345854)

27. Sinto R, Lie KC, Setiati S, et al. Blood culture utilization and epidemiology of antimicrobial-resistant bloodstream infections before and during the COVID-19 pandemic in the Indonesian national referral hospital. Antimicrob Resist Infect Control 2022; 11:73. (PMID: 35590391)

28. Gur D, Hasdemir U, Cakar A, et al. Comparative in vitro activity of plazomicin and older aminoglyosides against Enterobacterales isolates; prevalence of aminoglycoside modifying enzymes and 16S rRNA methyltransferases. Diagn Microbiol Infect Dis 2020; 97:115092. (PMID: 32569921)

29. Yeganeh Sefidan F, Mohammadzadeh-Asl Y, Ghotaslou R. High-Level Resistance to Aminoglycosides due to 16S rRNA Methylation in Enterobacteriaceae Isolates. Microb Drug Resist 2019;25: 1261-5. (PMID: 31211656)

30. Abo-State MAM, Saleh YE-S, Ghareeb HM. Prevalence and sequence of aminoglycosides modifying enzymes genes among E. coli and Klebsiella species isolated from Egyptian hospitals. *J Radiat Res Appl Sci* 2018;11:408-15. https://doi.org/10.1016/j.jrras.2018.08.005.

31. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268-81. (PMID: 21793988)

32. Kadri SS, Adjemian J, Lai YL, et al. Difficult-to-Treat Resistance in Gram-negative Bacteremia at 173 US Hospitals: Retrospective Cohort Analysis of Prevalence, Predictors, and Outcome of Resistance to All First-line Agents. Clin Infect Dis 2018;67:1803-14. (PMID: 30052813)

33. Lister PD, Wolter DJ, Hanson ND. Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev 2009;22:582-610. (PMID: 19822890)

34. Wolter DJ, Lister PD. Mechanisms of β-lactam resistance among Pseudomonas aeruginosa. Curr Pharm Des 2013;19:209-22. (PMID: 22894618).

35. Xie J, Yang L, Peters BM, et al. A 16-year retrospective surveillance report on the pathogenic features and antimicrobial susceptibility of Pseudomonas aeruginosa isolates from FAHJU in Guangzhou representative of Southern China. Microb Pathog 2017;110:37-41. (PMID: 28629721)

36. Copur Cicek A, Erturk A, Ejder N, et al. Screening of Antimicrobial Resistance Genes and Epidemiological Features in Hospital and Community-Associated Carbapenem-Resistant Pseudomonas aeruginosa Infections. Infect Drug Resist 2021;14:1517-26. (PMID: 33907430)

37. Zhang X, Gu B, Mei Y, Wen Y, Xia W. Increasing resistance rate to carbapenem among blood culture isolates of Klebsiella pneumoniae, Acinetobacter baumannii and Pseudomonas aeruginosa in a university-affiliated hospital in China, 2004-2011. J Antibiot (Tokyo) 2015;68:115-20. (PMID: 25182483)

38. Pachori P, Gothalwal R, Gandhi P. Emergence of antibiotic resistance Pseudomonas aeruginosa in intensive care unit; a critical review. Genes Dis 2019;6:109-19. (PMID: 31194018)

39. Garza-Gonzalez E, Morfin-Otero R, Mendoza-Olazaran S, et al. A snapshot of antimicrobial resistance in Mexico. Results from 47 centers from 20 states during a six-month period. PLoS One 2019;14:e0209865. (PMID: 30913243)