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Investigation of the frequency and antimicrobial susceptibility patterns of bacteria isolated from blood cultures in Khorramabad in 2018

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Abstract

Background: The widespread increase in the prevalence of antibiotic resistance in bacteria is considered one of the biggest problems in the global health and treatment system today. Therefore, this study was conducted to investigate the prevalence and antibiotic resistance pattern of bacteria isolated from blood cultures at Shahid Rahimi Hospital in Khorramabad in 2018.

Methods: This cross-sectional study investigated blood cultures of patients admitted to Shahid Rahimi Hospital from June to December 2018. The disc diffusion method was used according to CLSI instructions, and the data were analyzed using SPSS software.

Results: Out of a total of 2591 blood culture samples, 247 blood culture samples were positive. The highest frequency was related to the emergency department (62%) and ICU (23%), and the lowest frequency was related to the children's department (2.4%). Among the tested samples, 28% were Gram-negative bacilli and 72% were Gram-positive cocci. The most common Gram-negative and Gram-positive bacteria were *Escherichia coli* (38%) and *Staphylococcus epidermidis* (54.2%), respectively. Among Gram-negative bacteria, the highest level of resistance was seen in *Acinetobacter baumannii* strains, which showed resistance to all antibiotics. Among Gram-positive bacteria, the highest resistance of *Streptococcus* group D strains to the antibiotic erythromycin (92%) was reported.

Conclusion: The prevalence of antibiotic resistance in common microorganisms isolated from blood cultures in different departments is worrying, and choosing an effective drug to eliminate these bacteria by performing accurate laboratory tests is of particular importance.

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Introduction

The presence of bacteria in the blood (Bacteremia) is crucial and can lead to severe consequences and even death. Appropriate and prompt treatment of this infection is essential because it significantly reduces mortality (1). For the laboratory diagnosis of bacteremia, the most common method is the culture and isolation of bacteria, which is followed by the antibiotic sensitivity test of the isolated bacteria, which greatly helps in choosing the appropriate antimicrobial drug to treat the patient. The range of microorganisms that cause blood infection is different in different countries and also often varies from one treatment center to another within a country, region, or city. However, in most cases, Gram-negative bacteria contribute more to causing infections than Gram-positive ones, and nowadays sepsis reported from Gram-negative bacteria is increasing, especially in Asian countries (2,3). Common bacteria isolated from blood infections are *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter*, *Escherichia coli*, *Enterobacter*, coagulase-negative *Staphylococcus*, and *Staphylococcus aureus* (4). Investigation of antibiotic resistance is based on dilution and diffusion. The basis of the agar diffusion disc method is diffusion, which shows the resistance of bacteria to antibiotics. Relying on the data obtained from the results of determining antibiotic sensitivity and resistance, which is different according to the isolated bacterial species, helps doctors in choosing the appropriate antibiotic and the treatment process of the patients (5).

Due to the increase in the use of antibiotics, a large number of bacteria are resistant to antibiotics, and the process of treating blood infections has become a challenge (6,7). Research has shown that blood infection caused by antibiotic-resistant bacteria results in higher mortality, long-term hospitalization, and higher costs than antibiotic-sensitive bacteria (8). Some studies showed that the most common bacterial causes of blood infections are *S. aureus* and *P. aeruginosa* (9). In studies in Iran, it was also reported that the highest frequency of microorganisms isolated from patients with acquired blood infections caused by hospital infections is related to *E. coli* and *K. pneumoniae*. Also, imipenem was recognized as the most effective antibiotic against isolated strains (10).

This study aimed to investigate the prevalence and antibiotic resistance patterns of bacterial strains in the blood culture samples from Shahid Rahimi Hospital, in Khorramabad City (South West of Iran).

Methods

This study was designed as a cross-sectional study. After obtaining the necessary permissions from the honorable Vice President of Research at Lorestan University of Medical Sciences, the information recorded in this study was collected in Shahid Rahimi Hospital in Khorramabad for 7 months from June to December 2018. The sampling method used was consecutive and available.

Cultivation of samples and identification of bacteria

Initially, the samples were cultured on blood agar, chocolate agar, and Eosin Methylene Blue (EMB) agar at 24, 48, and 72 hours and on the seventh day after being kept in the incubator, and after incubation at 35 ±2°C, Gram staining was prepared from the resulting colonies. Standard microbial tests including oxidase, catalase, DNase, coagulase, OF fermentation, citrate consumption, sugars, indole, motility, and Methyl Red and Voges–Proskauer (MR-VP) tests were used to determine the species of bacteria.

Antimicrobial susceptibility testing

To evaluate the level of resistance of Gram-negative bacteria isolated from blood culture, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) and by the agar disk diffusion method, Mueller Hinton Agar culture medium (Merck, Germany) and antibiotic discs (PADTAN TEB, Iran) were used (11). For Gram-negative bacteria, Cefoxitin 30 µg, Cefepime 30 µg, Ceftriaxone 30 µg, Cefotaxime 30 µg, Ciprofloxacin 5 µg, Amikacin 30 µg, Gentamicin 10 µg, and Tetracycline 30 µg were tested. For Gram-positive bacteria, Cephalexin 30 µg, Penicillin 10 µg, Ciprofloxacin 5 µg, Levofloxacin 30 µg, Gentamicin 10 µg, Tetracycline 30 µg, Clindamycin 2 µg, and Erythromycin 15 µg were used (12).

Data analysis

SPSS software version 20 was used for statistical analysis and to check the frequency of the studied variables. Frequency ratios and percentages, as well as the mean and standard deviation of the data, were used to describe the information, and the results were presented in the form of statistical tables and graphs. Chi-square test (χ^2) was used to determine the relationship between dependent and independent variables. The significance level was considered less than 0.05.

Results

In the present study, 314 positive blood culture samples were isolated from a total of 2591 blood culture samples. Of these, 2 cases of fungi and 37 cases of Gram-positive bacilli (*Bacillus and Corynebacterium*) were excluded from the study as cases of blood culture contamination, as well as samples whose information was incomplete, and finally, 247 positive blood culture samples were included in the study. According to the results, most bacteria isolated from the positive blood culture samples were related to adults over 60 years old (40.1%) in 99 cases, and the least related to the age group between 11-20 years old (2.8%) with 7 cases. A total of 141 (57.1%) and 106 (42.9%) isolates were from men and women, respectively. The highest frequency of bacteria was reported in the emergency department (62%) and ICU (23%), and the lowest in the pediatric department (2.4%). The distribution and frequency of positive blood culture samples by department are shown in Table 1.

Among the 247-blood culture-positive samples, 70 samples (28%) were Gram-negative bacilli and 177 samples (72%) were Gram-positive cocci. The abundance of Gram-positive and Gram-negative bacteria in blood cultures based on different variables is shown in Table 2.

The abundance of bacteria isolated from blood cultures

The highest frequency of Gram-positive bacteria was related to *S. epidermidis* 96 (54%) and the lowest was related to Micrococcus spp. 9 (5%). Among the Gram-negative bacteria isolated, the highest frequency was *E. coli* 27 (38.5%) and the lowest was *A. baumannii* 3 (2.4%) (Figure 1).

Table 1. Distribution and frequency of positive blood culture samples by department

Sampling location unit	Relative frequency (%)	Absolute frequency (%)
Emergency	62	153
ICU	23	57
Internal	7.3	18
Surgery	3.3	8
Children	2.4	6
Other	2	5
Total	100	247

Results of antibiotic sensitivity and resistance patterns

In the present study, the highest and lowest resistance levels of *E. coli* were reported to cefotaxime (55%) and amikacin (1%), respectively. The highest and lowest resistance of *K. pneumoniae* to cefoxitin, ceftriaxone, and ciprofloxacin (62%), and to amikacin (12%) were reported. The highest and lowest levels of resistance of *Enterobacter aerogenes* were reported to cefoxitin, ceftriaxone, cefotaxime, and ciprofloxacin (40%), and resistance to cefepime, amikacin, gentamicin, and tetracycline (20%). The most resistance of *P. aeruginosa* strains was to tetracycline (75%), but none of the isolates were resistant to amikacin and gentamicin. *A. baumannii* strains were 100% resistant to cefoxitin, cefepime, ceftriaxone, cefotaxime, ciprofloxacin, amikacin, gentamicin, and tetracycline. Also, *Stenotrophomonas maltophilia* were 100% resistant to cefoxitin, cefepime, and ceftriaxone, and no resistance was reported to cefotaxime, amikacin, and gentamicin. Alkaligenes strains also showed resistance to ceftriaxone (83%) and the least resistance to tetracycline (16%). The highest and lowest levels of resistance of *Staphylococcus aureus* strains were to erythromycin (72%) and gentamicin (4%). The highest and lowest levels of resistance of *Staphylococcus epidermidis* were to clindamycin (70%) and gentamicin (30%). The highest and lowest levels of resistance of *Staphylococcus schleiferi* to clindamycin (66%) and penicillin (1%) were reported. The highest and lowest levels of resistance in *Staphylococcus haemolyticus* were observed to erythromycin (54%) and gentamicin and tetracycline (18%). Group D *Streptococcus* Gram-positive cocci showed the highest level of resistance to erythromycin (92%) and the lowest level to gentamicin (30%). *Micrococcal* strains had the highest resistance to erythromycin (66%) and no resistance was reported to ciprofloxacin, cephalexin, levofloxacin, and clindamycin (Figure 2).

Table 2. Frequency of Gram-positive and Gram-negative bacteria in blood cultures based on different variables

Variable	Property	Gram-negative bacilli	Gram-positive cocci	Total Number (%)	P-value
		Number (%)	Number (%)		
Age categories	1 >	9.12 (9)	6.26 (47)	22.7 (56)	< 0.006
	1-10	9.2 (2)	15 (5.8)	6.9 (17)	
	20-11	0 (0)	4 (7)	2.8 (7)	
	30-21	4.1 (1)	4 (7)	3.2 (8)	
	40-31	2.6 (8)	4.11 (11)	7.7 (19)	
	60-41	8.15 (13)	6.18 (28)	16.6 (41)	
	60 >	37 (35)	9.52 (62)	40.1 (99)	
Gender	Male	57.1 (49)	57 (101)	57.1 (141)	< 0.2
	Female	42.9 (30)	43 (76)	42.9 (106)	
Sampling location unit	Emergency	30 (48)	105 (70)	100 (153)	< 0.002
	ICU	23 (13)	77 (44)	57 (100)	
	Internal	34 (6)	66 (12)	100 (18)	
	Surgery	13 (1)	87 (7)	100 (8)	
	Children	17 (1)	83 (5)	100 (6)	
	Other	20 (1)	80 (4)	100 (5)	

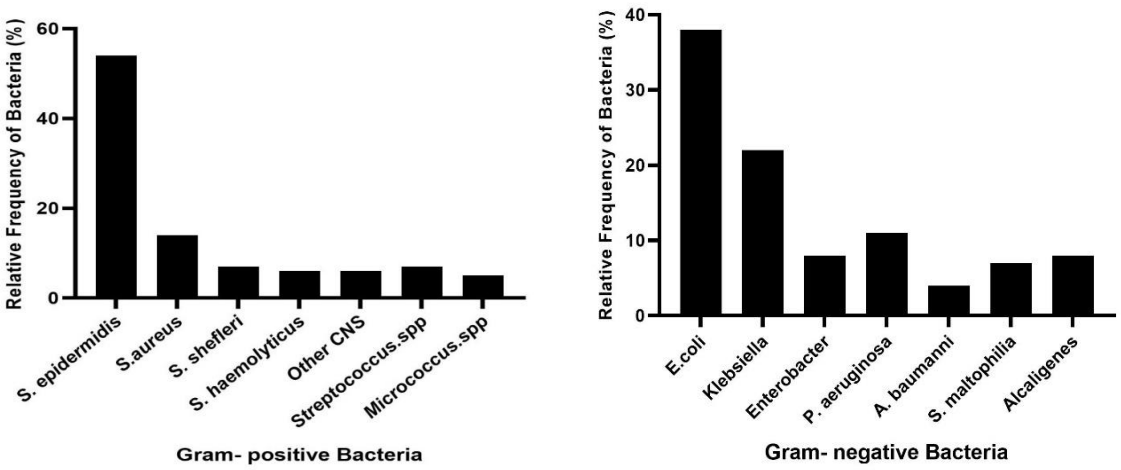

Figure 1. Abundance of Gram-positive and Gram-negative bacteria isolated from blood cultures

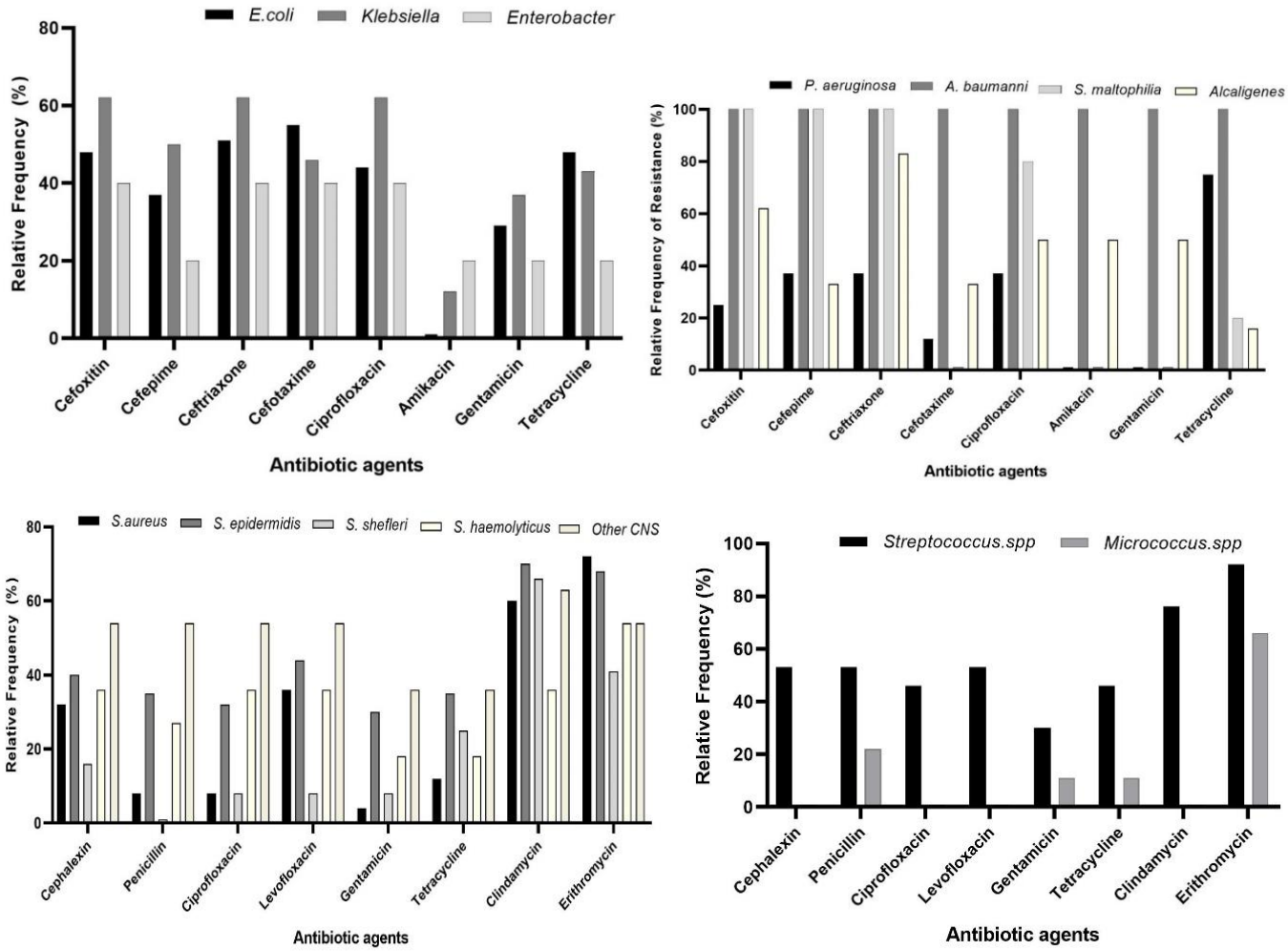

Figure 2. The amount of antibiotic resistance of gram-negative and gram-positive bacteria isolated from blood cultures of patients

Discussion

Antibiotic resistance is a phenomenon that can be seen more or less in different parts of the world (13,14). In general, bacteria may be naturally resistant to antibiotics or may have acquired resistance through mutation or acquisition of resistance genes from other bacteria, so the level of resistance in bacteria in different studies is different. These changes occur even in different years, depending on the factors that cause infection (15-18). In developed countries, Gram-positive bacteria are the most common cause of hospital bacteremia (19). Determining the organisms that cause blood infections, especially the hospital type, and quick and timely diagnosis of bacteremia are important in order to reduce mortality and treatment costs.

In our study, we found that the frequency of bacteria in the age group over 60 years was higher ($P<0.05$), which is probably due to the weakness of the immune system of these people due to old age. Also, the frequency of bacteria in the ICU department was higher than in other departments ($P<0.05$). Based on the findings, it has been determined that although only 5% of hospital beds belong to the ICU and less than 10% of all patients are treated in this department, more than 25-33% of hospital infections are dedicated to the ICU, and the use of mechanical ventilation, urinary catheters, and intravenous instruments are among the major factors that cause it (20,21). In our study, the frequency of Gram-positive cocci isolated from positive blood culture samples was reported in 177 samples (72%) and Gram-negative bacilli in 70 samples

(28%). The most Gram-positive cocci isolated from coagulase-negative *staphylococci* was 107 (60%), and among the coagulase-negative *staphylococci*, the most abundant isolated organism was *S. epidermidis* (54%). In the study of Mahmoudi et al., which examined 195 hospitalized patients with positive blood cultures in 1991-93, the highest frequency was related to infectious departments and children, respectively, and the most isolated organism was related to coagulase-negative *staphylococci*, which was responsible for 60 (30.77%) of cases (22). In the study of Mohammadi et al. in Sanandaj (2015), which was conducted on 180 positive blood culture samples that were isolated during one year, the highest frequency related to *S. epidermidis* was reported as 51.11% (11). By Tabatabai (2008), the most common bacteria among 104 positive blood cultures were coagulase-negative *staphylococci* (40.4%), followed by coagulase-positive *staphylococcus* (17.3%) (23). In many studies, *S. epidermidis* has been proposed as the most common cause of bacteremia, which is consistent with our study. Based on the research, about 50% of bacteremia is caused by Gram-positive cocci, and its prevalence is estimated at 330,000 to 700,000 cases annually in the United States. The increase in the prevalence of bacteremia caused by Gram-positive cocci in the last two decades is due to several factors such as the increase in the use of invasive diagnostic methods, which facilitate bacteria entering body tissues due to penetration into sterile areas without colonization (24).

In the present study, *E. coli* (38%) was the most common Gram-negative bacillus isolated from blood cultures. In the study of Vaez et al. in Iran, *E. coli* (29%) was reported as the most common Gram-negative bacillus causing bacteremia, which is consistent with our study (25). Also, in Iran, Mohammadi Mehr et al. reported the most abundant microorganisms isolated from patients with a blood infection caused by hospital infection as *E. coli* (35.6%) and *K. pneumoniae* (24.7%), respectively (26). Hsueh et al. in Taiwan (9), and Keihanian et al. in Rasht (27) reported that the most common Gram-negative bacillus causing blood infection was *P. aeruginosa*. The health level of the region, the time of the study, geographical differences, and the restriction of arbitrary use of antibiotics are also factors contributing to the differences in the results of the resistance level in bacteria in different geographical locations (28). In the present study, after the emergency department, the ICU had the highest rate of positive blood cultures. In our study, the highest and lowest resistance of *S. aureus* was to erythromycin (72%) and gentamicin (4%). The highest and lowest resistance in *E. coli* isolates were to cefotaxime (55%) and amikacin (1%). Among 107 dialysis patients in Tabriz, Akbarzadeh et al. isolated 50 *Staphylococcus* spp. and reported the highest sensitivity to gentamicin (80%) and ciprofloxacin (90%) (29). In the study of Nouri et al. in Hamedan on blood infections, the highest level of resistance of bacteria isolated from blood culture was related to *A. baumannii*, *S. epidermidis*, and *P. aeruginosa*, respectively (21). Each of the bacteria shows different sensitivity to different antibiotics in different regions and at different times, and the treatment of bacterial infections is completely related to the ability of bacteria to develop resistance to antimicrobial agents. Therefore, by determining the prevalence of microbial agents that cause blood infection and the pattern of antibiotic resistance annually, it is possible to help doctors and laboratory technicians to choose effective antibiotics for the purpose of antibiogram testing, because choosing effective antibiotics for treatment is necessary and leads to reduced treatment costs (30,31). It is also suggested that due to the high resistance of some isolated bacteria to antibiotics, regional and even national planning should be done to remove some antibiotics from the list of consumed antibiotics in order to witness a reduction in the rate of antibiotic resistance and better effective decision-making.

Conclusion

The prevalence of antibiotic resistance in bacteria isolated from blood cultures in the present study is significant. Choosing an effective and accurate antibiotic for treatment can have a significant impact on reducing costs and shortening the length of the treatment period.

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Ethical statement

The current research project was approved by the Research Ethics Committee of Lorestan University of Medical Sciences with the code of ethics IR.LUMS.REC.1399.340.

Conflicts of interest

The authors declare no conflict of interest, financial or otherwise.

Author contributions

Shakib P.: Writing the article and data review; Halimi Sh.: Data analysis; Mehrabnejad E.: Data collection; Rezaei F.: Scientific advisor; Delfani S.: Research design and project management.

Data availability statement

Data supporting our study findings are available from the corresponding author upon reasonable request.

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