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Identification of Pathogenic Bacteria in Blood Cultures and Susceptibility Testing of Isolates With Various Antibiotics

Mehdi Kholoujini,[1] Pezhman Karami,[2] Azad Khaledi,[3] Alireza Neshani,[3] Parastoo Matin,[4] and Mohmad Yosef Alikhani[2,*]

[1]Faculty of Medicine, Tarbiat Modares University, Tehran, IR Iran
[2]Department of Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran
[3]Antimicrobial Resistance Research Center, Avicenna Research Institute, Department of Microbiology and Virology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, IR Iran
[4]Microbiology Laboratory of Shahid Beheshti Hospital, Hamadan University of Medical Sciences, Hamadan , IR Iran

[*]*Corresponding author*: Mohmad Yosef Alikhani, Department of Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran. Tel: +98-9125443147, E-mail: alikhani43@yahoo.com

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Abstract

Background: Blood infections are an extensive range of disorders that can vary from limited bacteremia to fatal septicemia. Bacteremia refers to the transient presence of a bacterium in the bloodstream. A delay in the diagnosis and treatment of sepsis can cause mortality, with a 20% - 50% prevalence rate.
Objectives: Due to the changing patterns of antibiotic resistance, as well as differences in patterns over time in different settings, we decided to identify infectious agents and their antibiotic resistance patterns in blood cultures.
Materials and Methods: This study was conducted at Shahid Beheshti hospital, Hamadan, Iran, during a one-year period (March 21, 2014, to March 22, 2015). From patients with suspected bloodstream infections, 5-10 mL of blood was collected three times and inoculated into culture bottles. After identifying the types of microorganisms, susceptibility testing was performed according to CLSI standards, and the results were analyzed with statistical software.
Results: In the present study, 2,130 blood cultures were obtained from 710 patients (384 females and 326 males). Of these cultures, 232 (18.9%) were positive; 107 (46%) and 125 (54%) were from females and males, respectively. Most of the positive cultures were related to the internal medicine and hematology wards, which had 132 cases (56.9%), and the ICU, with 37 cases (16%). The most frequent isolates were *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter baumannii*, and coagulase-negative *Staphylococcus aureus*, with prevalence rates of 18.2%, 24.1%, 10.3%, and 10.3%, respectively. The most effective antibiotic against Gram-positive isolates was vancomycin.
Conclusions: This study revealed that the most effective antibiotics against two Gram-negative and Gram-positive groups were amikacin and norfloxacin, so it is recommended that these antibiotics be used empirically, at least in the setting where this study was conducted, before performing the culturing and antibiogram process.

Keywords: Antibiotic Resistance, Blood Culture

1. Background

Blood infections are an extensive range of disorders that can vary from limited bacteremia to fatal septicemia (1). If the presence of a bacterium is accompanied by its multiplication in the bloodstream, it is referred to as septicemia (2). Sepsis is one of the leading causes of mortality all over the world, and Iran is no exception (3). With delays in the diagnosis and treatment of sepsis, the mortality rate may increase up to 50% (4). Despite the high costs of sepsis and its treatment, the risk of death remains very high compared to other diseases (3). Disappointingly, in recent years, the number of sepsis cases has been rising significantly (5).

Studies show that a variety of microorganisms are capable of causing septicemia, which usually depends on factors such as different geographical location. For example, a study on microorganisms isolated from blood cultures in India reported that *S. aureus*, coagulase-negative *Staphylococci*, and *Enterobacter* are the most common causes of bloodstream infections, while a similar study in Iran showed that *Pseudomonas* and coagulase-negative *Staphylococci* were seen in blood cultures more often than others (1, 6).

One of the main challenges in the treatment of blood infections is the increasing resistance of bacteria to antibiotics (7). There are a variety of reasons for this increased resistance, the most important being the indiscriminate use of antibiotics; moreover, indiscriminate use of antibiotics can cause side effects, such as drug allergies, changes in the body's normal flora, and hiding serious infections without completely eliminating them (8). Selecting the most appropriate antibiotics to treat blood infections requires blood cultures followed by susceptibility testing. However, it should be noted that due to the urgency of blood infections and the requirement for prompt treatment, there is usually not enough time for culturing and susceptibility testing. Hence, according to existing guidelines, patients are first treated empirically with appropriate broad-spectrum antibiotics, and after the culture results and antibiotic susceptibility testing, the therapeutic method is revised. Unfortunately, studies show that approximately half of the empirical treatments prescribed by physicians in this situation are not consistent with the results of cultures and susceptibility tests, which leads to high antibiotic resistance (9). It is clear that the determination of the most common organisms isolated from blood cultures and the antibiotic-resistance patterns in each region can help to identify the best early antibiotic treatment for patients with bloodstream infections (10). This will enable physicians to prescribe appropriate antibiotics until receiving the culture and susceptibility test results, and will increase the cure rate and prevent inappropriate antibiotic use.

2. Objectives

Due to the changing patterns of antibiotic resistance, as well as differences in the patterns over time in different settings, we decided to identify infectious agents and their antibiotic-resistance patterns in blood cultures.

3. Materials and Methods

This cross-sectional study was conducted at Shahid Beheshti hospital, Hamadan, Iran, over a one-year period (March 21, 2014, to March 22, 2015). From patients with suspected bloodstream infections, 5-10 mL of blood was drawn three times and the samples were inoculated into culture bottles containing brain-heart infusion broth medium. The bottles were incubated at 37°C. In order to separate the bacterial isolates within 24 hours and for seven days, subcultures were done on blood agar and MacConkey agar. First, rapid tests for the detection of isolated bacteria were performed, such as Gram staining, catalase, and oxidase. Then, according to these results, as well as colony morphology and hemolytic type, the standard biochemical tests were performed to identify the genus and species of organisms (4, 11). To evaluate the antibiotic resistance of the isolates, the Kirby-Bauer disk diffusion test was used, based on CLSI protocols (12). The antibiotics applied in this study included trimethoprim-sulfamethoxazole, vancomycin, amikacin, ciprofloxacin, ceftriaxone, cefotaxime, ceftazidime, piperacillin, imipenem, cefazolin, norfloxacin, gentamicin, azithromycin, and methicillin. The diameter of the inhibition zone was measured with a ruler, and the results were compared with standard tables. Finally, the data were analyzed using SPSS software (version 19.0.0; SPSS Inc., Chicago, IL, USA), and significant differences in variables were analyzed by the χ^2 test.

4. Results

In the present study, 2,130 blood cultures were obtained from 710 patients (384 females and 326 males). Of these cultures, 232 (18.9%) were positive; 107 (46%) were from females and 125 (54%) were from males. Most of the positive cultures were related to the internal medicine and hematology wards, with 132 cases (56.9%), and to the ICU, with 37 cases (16%). The frequency of positive cultures from each ward are reported in Table 1. In this study, 14 types of microorganisms were isolated from the blood cultures. The most frequent isolates were *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter baumannii*, and *Staphylococcus aureus* (coagulase-negative), with prevalence rates of 18.2%, 24.1%, 10.3%, and 10.3%, respectively. Approximately 75% (175 cases) of isolated microorganisms were Gram-negative, as shown in Table 2. Approximately 25% (57 cases) of the isolated organisms were Gram-positive, the most frequent of which were *Staphylococcus* species (19.8%). The Gram-positive microorganisms are presented in Table 3. The most effective antibiotic against Gram-positive isolates was vancomycin. The pattern of antibiotic resistance in the Gram-negative group showed that in *P. aeruginosa* isolates, the highest resistance was to trimethoprim-sulfamethoxazole (SXT) (87.5%) and ceftriaxone (71.4%), while the lowest resistance was to imipenem (16%) and amikacin (19.6%).

Table 1. Frequency of Positive Cultures in All Samples

Ward	N(%)
Internal medicine and hematology	132 (56.9)
ICU	37 (16)
Surgery	20 (8.6)
Pulmonary	19 (8.2)
Intestinal	14 (6)
Nephrology	10 (4.3)

Table 2. Frequency of Gram-Negative Bacteria Isolates from Blood Cultures

Type of Bacteria	N (%)
P. aeruginosa	56 (24.1)
E. coli	42 (18.2)
A. baumannii	24 (10.3)
K. pneumoniae	20 (8.6)
Citrobacterspp.	11 (4.8)
Enterobacterspp.	10 (4.3)
Serratiaspp.	5 (2.1)
Alcaligenes faecalis	3 (1.3)
Salmonella typhi	2 (0.86)
Proteus vulgaris	2 (0.86)
Total	175 (75.42)

Table 3. Frequency of Gram-Positive Bacteria Isolates from Blood Cultures

Type of Bacteria	N(%)
CNS	24(10.3)
S. aureus	22(9.5)
Viridans streptococci	8(3.5)
Enterococcus spp.	3(1.3)
Total	57(24.6)

Abbreviation: CNS, Coagulase-negative *Staphylococcus*.

The *E. coli* isolates in our study also showed the most resistance to SXT (88%) and cefazolin (76.1%), while the lowest resistance was to imipenem (9.5%) and piperacillin (16.6 %). In *A. baumannii*, the highest resistance was to cefazolin (95.8%) and SXT (91.7%), and the lowest resistance was to imipenem (25%) and norfloxacin (37.5%). It is clear that resistance to SXT among the three main Gram-negative bacteria isolates (*P. aeruginosa*, *A. baumannii*, and *E. coli*) was very high, and most of the lowest resistance among these three bacteria was to imipenem (Table 4). This pattern of antibiotic resistance showed that the most effective antibiotics against Gram-positive isolates were vancomycin and norfloxacin, located at the next level. The highest antibiotic resistance of *Staphylococcus* was to SXT (> 50%). Methicillin-resistant *S. aureus* (MRSA) accounted for 40.9% of the *S. aureus* strains in this study. The antibiotic resistance of the Gram-positive microorganisms is shown in Table 5.

5. Discussion

Among all of the suspicious samples examined in this study, the frequency of positive blood cultures was 9.18%, compared to 4% - 18% in other similar studies (6, 13, 14). The rates of positive blood cultures in different geographic areas vary; for example, a frequency of 16% - 44% has been reported in several studies in India (15-17). In the present study, 75.4% of the isolates were Gram-negative and 24.6% were Gram-positive. The Gram-negative rate varies in studies conducted in Iran, from 33% reported by Deiham (18) to 85% reported by Saderi et al. (6). In most studies conducted in Asia, the highest prevalence was related to Gram-negative bacteria, consistent with our results (4, 6, 13, 15-18). Also, a study conducted by Shahidi et al. over three years found that *S. aureus*, *Enterobacter*, and *Pseudomonas*, respectively, were the most common bacteria (13).

Although the frequency of the assortment of organisms from blood cultures is usually different among studies, the types of organisms isolated are usually fixed. The present study isolated 14 types of organisms, almost identical to those isolated in other studies, differing only in prevalence (4, 6, 13, 15-18). In this study, the most effective antibiotics against *P. aeruginosa* were imipenem, amikacin, and norfloxacin. Arora et al. also reported that amikacin and ciprofloxacin had the greatest impact against *Pseudomonas*, which is similar to our results (1). In studies conducted at different times and in different settings, the most effective antibiotics against *Pseudomonas* were usually fluoroquinolones (1, 6, 13, 17, 18). Also in this study, for the second most prevalent Gram-negative strain, *E. coli*, the greatest resistance was to co-trimoxazole, corresponding to a similar study in Dezful, Iran (18). The prevalence of MRSA in this study was 40.9% compared to an average prevalence of 52.7% in Iran (19). Our survey showed that the most effective antibiotics against Gram-positive bacteria were vancomycin (resistance rate 0%) and norfloxacin (resistance rate 16.85%). Most similar studies in Iran have reported that the most effective antibiotics against Gram-positive bacteria, especially staphylococci isolated from blood, were vancomycin and fluoroquinolones (6, 13, 18). Antibiotic resistance in the Gram-positive bacteria isolated in this study was relatively low, and the highest resistance in all Gram-positive isolates was to co-trimoxazole (60.57%); resistance to other antibiotics was approximately < 50%. The final aim of this study was to identify effective antibiotics against agents of blood infection, to be applied as empirical treatment before receiving culture and antibiogram results. This work will lead to more effective treatment of these infections, while reducing the risk of antibiotic resistance.

Table 4. Antibiotic Resistance Among Gram-Negative Microorganisms[a]

Type of Bacteria	CP	IPM	CZ	NOR	CAZ	GM	AN	PIP	SXT	CTX	CRO
P. aeruginosa	14 (25)	9 (16)	4.6 (82.1)	12 (21.4)	19 (33.9)	21 (37.5)	11 (19)	12 (21.4)	49 (87.5)	30 (53.5)	40 (71.4)
E. coli	23 (55)	4 (9.5)	32 (76.1)	20 (47.6)	24 (57.1)	17 (40.4)	8 (19)	7 (16.6)	37 (88)	25 (59.5)	26 (61.9)
A. baumannii	10 (41.6)	6 (25)	23 (95.8)	9 (37.5)	21 (87.5)	19 (79.1)	16 (66)	18 (75)	22 (91.7)	21 (87.5)	21 (87.5)
K. pneumoniae	9 (45)	4 (20)	14 (70)	6 (30)	13 (65)	12 (60)	7 (35)	5 (25)	18 (90)	13 (65)	14 (70)
***E.* spp.**	1 (10)	0 (0)	4 (40)	0 (0)	2 (20)	3 (30)	2 (20)	0 (0)	5 (50)	2 (20)	3 (30)
***C.* spp.**	5 (45.4)	1 (9)	7 (63.6)	2 (18.1)	4 (36)	5 (45)	2 (18)	2 (18.1)	9 (81.8)	6 (54.5)	7 (63.6)
***S.* spp.**	1 (20)	0 (0)	3 (60)	0 (0)	2 (40)	1 (20)	0 (0)	0 (0)	4 (80)	3 (60)	3 (60)
S. typhi	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)
P. vulgaris	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)
A. faecalis	1 (33.3)	0 (0)	1 (33.3)	0 (0)	1 (33.3)	1 (33.3)	0 (0)	0 (0)	2 (66.6)	1 (33.3)	2 (66.6)

Abbreviations: AN, amikacin; AZM, azithromycin; CAZ, cefazolin; CP, ciprofloxacin; CRO, ceftriaxone; CTX, cefotaxime; CZ, ceftazidime; GM, gentamicin; IPM, imipenem; ME, methicillin; NOR, norfloxacin; PIP, piperacillin; SXT, trimethoprim-sulfamethoxazole; V, vancomycin.
[a]Values are presented as No. (%).

Table 5. Pattern of Antibiotic Resistance Among Gram-Negative Bacteria[a]

Type of Bacteria	CZ	CRO	AZM	AN	CP	NOR	CTX	SXT	V	GM	ME
S. aureus	11 (50)	7 (31.8)	5 (22.7)	6 (27.2)	5 (22.7)	2 (9.1)	6 (27.2)	13 (59.1)	0	10 (45.4)	9 (40.9)
CNS	8 (33.3)	10 (41.6)	11 (45.8)	4 (16.6)	8 (33.3)	3 (12.5)	5 (20.8)	13 (54.1)	0	9 (37.5)	4 (16.6)
***Enterococcus* spp.**	2 (66.6)	1 (33.3)	1 (33.3)	1 (33.3)	2 (66.6)	1 (33.3)	1 (33.3)	2 (66.6)	0	3 (100)	1 (33.3)
Viridans streptococci	3 (37.5)	4 (50)	1 (12.5)	1 (12.5)	3 (37.5)	1 (12.5)	2 (25)	8 (62.5)	0	3 (37.5)	2 (25)

Abbreviations: AN, amikacin; AZM, azithromycin; CP, ciprofloxacin; CRO, ceftriaxone; CTX, cefotaxime; CZ, ceftazidime; GM, gentamicin; ME, methicillin; NOR, norfloxacin; SXT, trimethoprim-sulfamethoxazole; V, vancomycin.
[a]Values are presented as No. (%).

5.1. Conclusion

The results of our study showed that the most effective antibiotics against two Gram-negative and Gram-positive groups were amikacin and norfloxacin. It is therefore recommended that these two antibiotics be used empirically in the setting in which this study was conducted, before performing the culturing and antibiogram process.

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Footnote

Authors' Contribution: Study concept and design: Mehdi Kholoujini and Pezhman Karami; drafting of manuscript: Azad Khaledi and Alireza Neshani; critical revision of the manuscript for important intellectual content: Mohmad Yosef Alikhani; performance of laboratory tests: Parastoo Matin.

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