Bacterial Distribution and Susceptibility in Bloodstream Infection in Primary-Care Hospital in Nova Friburgo, Rio de Janeiro

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Abstract

Human blood is sterile. When there is an infection, microorganisms can reach the bloodstream, which is known as bacteremia. This can be detected by blood culture. To study the prevalence of microorganisms isolated from blood culture samples. One hundred thirty-seven blood cultures were collected from hospitalized patients. All blood samples were processed for culture using a BACT/Alert blood culture machine. Further identification of bacterial pathogens and their antimicrobial susceptibility test were performed using standard microbiological procedures.

Among these samples, 20.44% had a positive culture, 60.71% of which belonged to females and 39.29% were from male patients. The most common microorganisms found in positive blood cultures were coagulase negative *Staphylococcus* (33.33%), *Staphylococcus aureus* (23.33%), *Klebsiella pneumoniae* (6.70%), and *Escherichia coli* (6.66%). One sample showed an ESBL-producing *E. coli*. Gram-positive microorganisms were 100% susceptible to vancomycin and displayed considerable resistance to oxacillin. Many Gram-negative bacteria were resistant to cephalosporins.

Introduction

Bloodstream infections (BSI) have been noted to be among the top 7 causes of mortality in Europe and North America [Waters at al, 2017]. BSI acquired in hospitals are generally associated with significant patient morbidity and mortality worldwide and can trigger septic shock with multiple organ failure, which increases mortality, time and hospitalization costs [Riu et al, 2017].

Bacteria from the patient’s microbiota, professionals, surfaces and hospital materials that are not sanitized or sterilized properly can cause serious infections of the bloodstream. A blood culture is the laboratory test that allows the isolation and identification of the etiological agents involved in BSI. In addition, an antibiotic susceptibility test can be performed from positive blood cultures, which contributes to the fast implementation of appropriate antimicrobial therapy.

Gram-positive cocci (GPC) are usually the most prevalent bacteria, but BSIs related to Gram-positive bacilli (GNB) are associated with higher mortality rates. The main related pathogens are coagulase-negative *Staphylococcus*, *Staphylococcus aureus*, Enterobacteria and Gram-negative bacilli, such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. [Riu et al 2017; Brixner et al, 2019]

In recent years, antibiotic resistance has spread rapidly around the world and poses a critical threat to public health. [Gastmeier et al, 2018] A worrying fact that has long been discussed is the increase in the isolation rate of multidrug-resistant microorganisms, which reduces the available therapeutic options and affects patient care. The main hospital pathogens have the ability to adapt perfectly to current health care. The ability of multiple virulence characteristics and a wide resistance of antimicrobial agents through expression of efflux pumps, mutation of the target gene, enzymatic modification, among other mechanisms made as modifications by these highly fatal microorganisms. [Ayoub et al, 2020].
problems of nosocomial infections and the increase in antimicrobial resistance are highly relevant and should not be overlooked [Gastmeier et al, 2018].

The aim of this study was to identify the bacterial pathogens causing major bloodstream infections in a primary-care hospital in Nova Friburgo, Rio de Janeiro and determine their antibiotic susceptibility pattern.

**Materials And Methods**

In this study, 137 blood samples were obtained from patients attending services at a primary-care hospital in Nova Friburgo, Rio de Janeiro, Brazil. All blood samples were processed for culture using a BACT/Alert blood culture machine to identify the presence of bacterial pathogens.

Collected blood samples were directly inoculated into blood culture bottles. Bottles were incubated in the BACT/Alert machine for up to 5 days. Positive culture samples were directly inoculated onto Mac Conkey (MC) agar, chocolate agar and blood agar (5% sheep blood) plates and incubated at 35 °C under aerobic conditions. Chocolate and blood agar plates were incubated at 35 °C under microaerophilic conditions (containing 5% CO2). Bacterial pathogens were identified using standard bacteriological procedures [Simundic, et al, 2015].

Antimicrobial susceptibility tests were performed by using the disk diffusion method, and susceptibility patterns were determined following CLSI guidelines. Antibiotic susceptibility was tested for CN (10 μg), SXT (25 μg), Cip (5 μg), CRO (30 μg), AMP (10 μg), Caz (30 μg), Imp (10 μg), Net (30 μg), Ak (30 μg), CFM (5 μg), Azi (15 μg), Pen G (10 μg), E (15 μg), C (30 μg), Van (30 μg) and Tet (30 μg). We were not able to find any standard definition of multidrug resistance (MDR) and observed that many previous studies have used the definition of MDR as resistance against three or more classes of antibiotics [Simundic, et al, 2015].

**Results**

One hundred thirty-seven blood cultures were analyzed in this study. Among these, 79.56% (109) were negative and 20.44% (28) were positive (Figure 1).

Among the positive cultures, 60.71% (17) were from females and 39.29% (11) from males (Figure 2).

Two mixed cultures were identified, both from female patients.

Gram-positive bacteria (GPB) were present in 60% (18) of the positive blood cultures, while Gram-negative bacilli (GNB) were isolated from 26.66% (8) of the samples. Yeast was found in 13.34% (4) of the samples (Figure 3).

Among the isolated microorganisms, coagulase-negative *Staphylococcus* (CoNS) was the most prevalent, present in 33.33% (10) of the cultures. *S. aureus* was the second most common, present in 23.33% (7) of
the samples. The presence of a strain of ESBL-producing *E. coli* was observed (Table 1).

| Microorganisms                                      | Nº of isolated microorganisms | Percentage (%) |
|-----------------------------------------------------|-------------------------------|----------------|
| *Enterobacter sp.*                                   | 1                             | 3.33           |
| *Serratia marcescens*                                | 1                             | 3.33           |
| *Staphylococcus aureus*                              | 7                             | 23.33          |
| Coagulase-negative *Staphylococcus*                  | 10                            | 33.33          |
| Yeast                                               | 4                             | 13.33          |
| *Aeromonas sp.*                                      | 1                             | 3.33           |
| *Klebsiella pneumoniae*                              | 2                             | 6.70           |
| *B-hemolytic Streptococcus* not group A and not group B* | 1                             | 3.33           |
| *Proteus sp.*                                        | 1                             | 3.33           |
| *Escherichia coli* (1 ESBL-producing *E. coli)*      | 2                             | 6.66           |
| **Total**                                            | **30**                        | **100**        |

In relation to the susceptibility and resistance profile, it was noted that only 50% of the microorganisms were susceptible to amoxicillin + clavulanic acid. All microorganisms were susceptible to vancomycin and rifampicin (Table 2).
In general, BGN were resistant to first- and second-generation cephalosporins.

CoNS was 100% susceptible to rifampicin, vancomycin, and teicoplanin. However, 62% of these were resistant to oxacillin. In addition, quinolones (ciprofloxacin and levofloxacin) showed good activity against the CNS (Table 3).

| Antibiotics                        | Susceptibility | Resistance |
|-----------------------------------|----------------|------------|
| Ciprofloxacin                     | 62.5           | 37.5       |
| Levofloxacin                      | 57.15          | 42.85      |
| Amikacin                          | 76.19          | 23.81      |
| Cefepime                          | 76             | 24         |
| Gentamicin                        | 76.20          | 23.80      |
| Amoxicillin + Clavulanic Acid     | 50             | 50         |
| Cephalothin                       | 52.63          | 47.37      |
| Rifampicin                        | 100            | 0          |
| Vancomycin                        | 100            | 0          |
| Oxacillin                         | 43.75          | 56.25      |
**Table 3. Antibiotic susceptibility of coagulase-negative staphylococci recovered from blood cultures**

| Antibiotics            | Susceptibility (%) | Resistance (%) |
|------------------------|---------------------|----------------|
| Ciprofloxacin          | 44.4                | 55.6           |
| Levofloxacin           | 50                  | 50             |
| Amikacin               | 83.3                | 16.7           |
| Cefepime               | 62.5                | 37.5           |
| Gentamicin             | 75                  | 25             |
| Amoxicillin + Clavulanic Acid | 66.6              | 33.4           |
| Cephalothin            | 87.5                | 12.5           |
| Rifampicin             | 100                 | 0              |
| Vancomycin             | 100                 | 0              |
| Teicoplanin            | 100                 | 0              |
| Clindamycin            | 40                  | 60             |
| Oxacillin              | 37.5                | 62             |

*S. aureus* was 100% susceptible to amikacin, rifampicin, and vancomycin. The oxacillin resistance rate was 57.14% (Table 4).

**Discussion**

In this study, we aimed to identify the most prevalent pathogenic organisms involved in bloodstream infections (BSI) in patients in a primary-care hospital in Nova Friburgo, Rio de Janeiro. We also aimed to determine the antimicrobial susceptibility of the isolated pathogens against multiple antibiotics to observe trends of change in the profile of susceptibility to antibiotics.

During the last few years, BSI has become a major cause associated with increased morbidity and mortality rates. Microbial resistance against different antimicrobial agents has also increased [Riu et al, 2017]. The appearance of multiresistant microorganisms makes treatment more complicated [Gastmeier et al, 2016].

Gram-positive bacteria pose a serious threat due to the increased incidence of BSI worldwide. The problem is greatest in hospitals where methicillin-resistant *S. aureus* (MRSA) is considered a major health
threat [Zhu et al, 2018; Doernberg et al 2017]. In this study, we observed a higher rate of isolation of Gram-positive bacteria. These data can be attributed to the patient's greater access to health facilities where BSI by Gram-positive bacteria is common.

We identified Staphylococcus. aureus and coagulase-negative Staphylococcus (CoNS) as the two main gram-positive pathogenic bacteria associated with BSI in Nova Friburgo, Rio de Janeiro. Coagulase-negative staphylococci are one of the major opportunistic pathogens, and the incidence of CoNS bacteraemia is increasing.

This study found a higher prevalence of Gram-positive bacteria (GPB), confirming previous studies [Zhu et al, 2018, Rui et al 2019]. It is valid to say that, as mentioned, this prevalence may vary according to region, and therefore, some studies report a higher prevalence of Gram-negative bacilli (GNB) [Ahmed et al, 2017, Toyofuku et al, 2019].

This variation can be explained by several factors, such as an increase in the practice of invasive procedures, prophylactic treatment with antimicrobials that have high activity against GNB and have little or no activity against GPB, greater recognition of CoNS as pathogens, immunological status of the patient, artificial climate of the hospital, hand hygiene of health professionals, and hospital service profile.

Regarding the antimicrobial susceptibility profile, it was found that oxacillin did not have good activity against S. aureus and CoNS, which is dangerous because it is the drug of choice for the treatment of staphylococcal infections. One hundred percent of GPB were susceptible to rifampicin, vancomycin, and teicoplanin, drugs known for their great action against oxacillin-resistant strains [Hasani, 2013].

Although Escherichia coli (E. coli) is an important cause of bloodstream infection, few studies have described the epidemiology of this infection. E. coli strains generally produce extended-spectrum β-lactamase (ESBL), as well as determinants of quinolone resistance. In this study, we observed that first- and second-generation cephalosporins did not show good activity against GNB. The presence of this enzyme is a universal problem and occurs mainly due to the indiscriminate use of broad-spectrum cephalosporins in the hospital environment [Malande et al, 2019; Brixner et al, 2019].

The drugs of choice for the treatment of infections caused by ESBL-producing microorganisms belong to the carbapenem class, but use must be moderate and reasonable because resistance to this antimicrobial class has already been described [Karaiskos and Giamarellou, 2020].

The current pattern of antimicrobial resistance emphasizes the importance of creating national and international monitoring programs to prevent the spread of multidrug-resistant strains.

**Conclusion**

This study confirms the increase in prevalence of Gram-positive bacteria, which has been reported in various regions of the world and corroborates the literature regarding Gram-negative bacilli’s resistance
to first- and second-generation cephalosporins.

We did not have access to clinical data of patients, so some results may have been overestimated. More studies are needed to better evaluate the different features related to BSI.

Declarations

Conflict of interest statement

The local institutional council approved this study. For this type of study, formal consent is not applicable. The authors declare that they have no conflicting interests.

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**Figures**
Figure 1
Positive and negative blood cultures collected in primary care hospitals

Figure 2
Prevalence of positive samples in males and females.
Figure 3

Prevalence of positive blood cultures related to Gram positive bacteria, Gram negative bacteria, and yeast.