BACTERIOLOGICAL PROFILE AND DRUG RESISTANCE PATTERNS OF
BLOOD CULTURE ISOLATES AT A SECONDARY CARE HOSPITAL IN
BRAZIL

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Subject: SUBMISSION OF NEW MANUSCRIPT FOR EVALUATION

Dear Editor,

I am enclosing herewith a manuscript entitled “BACTERIOLOGICAL PROFILE AND DRUG RESISTANCE PATTERNS OF BLOOD CULTURE ISOLATES AT A SECONDARY CARE HOSPITAL IN BRAZIL” for publication in Brazilian Journal of Infectious Diseases as an Original Article.

This project was approved by the Ethics Comitee of Instituto de Medicina Integral Prof. Fernando Figueira (CEP-IMIP nº 3659-13) and received a financial support from PIBIC-CNPq / IMIP. With the submission of this manuscript I would like to undertake that the above mentioned manuscript has not been published elsewhere, accepted for publication elsewhere or under editorial review for publication elsewhere; and that my Institute’s representative is fully aware of this submission. I also confirm that the manuscript content represents the views of the coauthors.
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ABSTRACT

Objective: The purpose of this study is to analyze the most prevalent microorganisms causing bacteremia and their resistance profiles in a secondary care hospital in Pernambuco, Brazil.

Methods: This was a transversal, descriptive study, based on positive blood cultures collected from patients admitted in the hospital from January to December of 2012.

Results: About 1056 bacteria were isolated from 915 blood cultures (86% monomicrobial and 14% polymicrobial). The most prevalent microorganisms were coagulase-negative Staphylococci (53.2%), Staphylococcus aureus (10.2%), Acinetobacter baumannii (7.9%), Klebsiella pneumoniae (5.1%) and Enterococcus faecalis (3.5%). Coagulase-negative Staphylococci were oxacillin-resistant in 83.1% and S. aureus was in 61.1% of cases, but both were 100% susceptible to vancomycin and linezolid. There was no vancomycin resistant Enterococcus spp. Enterobacteriaceae were multidrug resistant in 30.2% of cases, and there was extended-spectrum beta-lactamas and carbapenemase production in 12.6% and 13.6%, respectively. K. pneumoniae was multidrug resistant in 59.3%, and produced extended-spectrum beta-lactamas in 42.6% and carbapenemase in 16.7%. Almost 87% of Acinetobacter baumannii isolates were multidrug resistant, and imipenem resistance rate was 81.2%. Except for Serratia marcescens, no other gram negative was resistant do polymyxin B.

Discussion: High frequencies of drug resistance were found among the most frequent microorganisms. These strains are susceptible only to a few broad spectrum antibiotics, which leaves limited options for treatment in critically ill patients.

Keywords: Bacteremia, drug resistance, anti-bacterial agents.
Introduction

Nosocomial infections are an important cause of morbidity and mortality throughout the world, increasing length of hospital stay and costs (1-3). Bloodstream infections (BSI) correspond to 9.9-14.2% of nosocomial infections (4, 5), and it is often necessary to initiate an empirical antibiotic treatment in critically ill patients before the cultures results are available. However antimicrobial therapy can exert selective pressure, and the indiscriminate use of antibiotics may lead to multidrug resistance(6). There have been reports of multidrug resistant strains of the most common nosocomial pathogens, such as *Staphylococcus aureus* (*S. aureus*), *Enterococcus spp.*, *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Acinetobacter baumannii* (*A. baumanii*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) (7-9).

The prevalence of pathogens and their resistance patterns may vary in different hospitals (10). Therefore knowing the most frequent pathogens becomes necessary for effective antibiotic treatment, decreasing length of hospital stay, morbidity and mortality of patients (11). Thus, the purpose of this study is to analyze the most prevalent microorganisms causing bacteremia and their resistance profiles in a secondary care hospital in Pernambuco, Brazil.

Materials and methods

Design and setting

This was a transversal, descriptive study, based on positive blood cultures collected from patients admitted in a public secondary hospital in Pernambuco, Brazil, from January to December of 2012. The hospital has 180 beds, distributed in five sectors: Intensive care unit (29), emergency (34), clinical ward (30), general surgery ward (29) and orthopedic ward (58).
Sample collection and processing

The blood samples were cultured by automated methods, using Bactec FX, and the microbiological identification and antimicrobial susceptibility were performed using Vitek 2 automated system. Each pathogen was classified as susceptible, intermediate or resistant to each antibiotic tested. Enterobacteriaceae were also tested for extended-spectrum beta-lactamases (ESBL) and classified as probable carbapenemase producers or not.

Analysis

It was included in the study all positive blood cultures in the hospital in the year of 2012, except those positive for fungus or mycobacteria. There were 915 positive samples and 1056 isolates. Multidrug resistance was defined as non-susceptibility to at least one agent in three or more antimicrobial categories, and oxacillin-resistant *S. aureus* isolates were categorized as multidrug resistant (MDR) as well (12). The isolates were also categorized by the hospital sector: ICU, wards and emergency.

Results

About 1056 bacteria were isolated from 915 blood cultures, from which 86% were monomicrobial and in 14% were polymicrobial. The most prevalent microorganism was coagulase-negative staphylococcus (CoNS), performing 53.2% of the total, followed by *S. aureus* (10.2%), *A. baumannii* (7.9%) and *K. pneumoniae* (5.1%). The prevalence of the most common microorganisms is shown in Figure 1.

The rates of resistance to carbapenems and cephalosporins were considerable among gram negative bacilli (GNB) (Table 1). Both *A. baumannii* and *S. marcescens* were
resistant to carbapenems and cephalosporins in more than 80% of cases, but they were mostly susceptible to gentamicin. *P. aeruginosa* was resistant to cepthazidimin 45.5% of cases, but only 13.6% of the isolates tested for piperacillin-tazobactam were resistant. Except for *S. marcescens*, no other GNB was resistant do polymyxin B. Gram positive cocci (GPC) were all susceptible to vancomycin and linezolid. There was a high frequency of oxacillin-resistant CoNS(83.1%) and *S. aureus* (61.1%) (Table 2)

The frequency of MDR GPC is shown in Figure 2, and the percentages of multidrug resistance, ESBL and carbapenemase producers among GNB are shown in Figure 3. High rates of multidrug resistance are shown among the most common bacteria, especially *A. baumannii*, *S. marcescens*, CoNS and *S. aureus*.

The ICU was responsible for 49.3% of isolates, emergency for 38.4% and wards for 12.2%. The most prevalent isolate is CoNS in all three sectors, but the frequency of the other pathogens varied between them. *A. baumannii* was more prevalent in ICU and wards comparing to emergency, while *S. aureus* was less prevalent in ICU. Gram negative bacteria were less frequent in emergency comparing to wards and ICU (Table 3).

**Discussion**

Although there have been an increase in gram negative bacteria reported in several studies (5, 13, 14), there is still a prevalence of gram positive organisms in some of them.(4, 15-17)Our study shows similar results, in which 70.8% of bacteria are gram positive versus 29.2% of gram negative. The most prevalent isolates were CoNS (53.2%), *S. aureus* (10.2%), *A. baumannii* (7.9%) and *K. pneumoniae* (5.1%). Although *P. aeruginosa* is an important pathogen reported in many studies, it represented only 2.1% of all isolates in our study.
A multicenter study performed in the US and another one in Europe also found CoNS as the most common bacteria, as well as studies in Colombia and India, but in lower frequencies (16.7-39.6%)(4, 5, 14, 17, 18). Other studies showed S. aureus as the most common bacteria, including a multicenter study performed in Brazil(13, 15). High levels of oxacillin resistant CoNS were found in our study (83.1%), but this founding is in consonance with results found in Brazil (13, 19) and other parts of the world (14, 20).

The high prevalence of CoNS could be explained by the fact that these bacteria are often skin colonizers, and the use of external devices predispose to infection caused by them.(21) This group is also the most common cause of contamination of samples, and it is necessary clinical correlation to discriminate whether it is a pathogen or a contaminant(22, 23).

Staphylococcus aureus was resistant to oxacillin in 61.1% of the isolates, similar to other recent studies – varying from 33.4 to 70.6% (13, 15, 16, 18, 24, 25). Because of the high rates of resistance, vancomycin should be considered as a first choice in critically ill patients who need empiric antimicrobial therapy, since there was no isolate resistant do this drug in our study.

Enterococcus spp. was observed in 4.8% of all isolates. There was no vancomycin resistant Enterococcus spp. in this study, in contrast to other studies showing different rates of resistance. A nationwide surveillance study in the US found 60% of vancomycin resistant E. faecium, but only 2% of resistant E. faecalis(18), while other studies show rates up to 29% (26-28).

The prevalence of A. baumannii in this study was 7.9%, and most of them were isolated either in the ICU or in wards, and only 2.9% of them were isolated in emergency. That could be explained by the fact that this species is associated with mechanic ventilation(7), and a significant amount of patients in wards in this hospital comes from the ICU. Almost
87% of the isolates were MDR, and imipenem resistance rate was 81.2%, results in conformity with a global tendency of increasing resistance rates (29-31).

A multicentric surveillance study in America was published in 2004, demonstrating carbapenem resistance rates ranging from 0.7-20% (20). Recent studies have shown that this is no longer a reality: 83% of A. baumannii isolates in a study in India were resistant to imipenem (14), similar to our results, 49.5% in Colombia (17), 55.9% in Brazil (13), 54% in Italy (32). This scenario has origin in multiple factors, including the indiscriminate use of carbapenems, and the fact A. baumannii is able to develop multiple resistance mechanisms (33).

Although the SENTRY study reported Latin America to register the higher rates of ESBL production, reaching 23.9% (20), in this study, there were 12.6% of ESBL producers among Enterobacteriaceae. Similar results were found in a study in Colombia, in which 14.7% were ESBL producers in blood, urine and other samples (34). Other studies in Brazil revealed rates from 22 to 29% (13, 35). Escherichia coli and K. pneumoniae were ESBL producers in 34.6% and 46.2% of the isolates respectively. Similar rates were described in a global surveillance, in which rates around 40% for E. coli and over 50% to K. pneumoniae were published (36).

Carbapenemase production was suspected in 13.6% of all Enterobacteriaceae. Enterobacter spp. and S. marcescens had the highest rates: 46.2% and 87.5%, while carbapenemase production in K. pneumonia was 16.7%. Carbapenem resistance rates seem to be very different in many studies. This founding was high compared 1.1% total carbapenem resistance found in a large study performed in USA (9) and to 1.3% found among K. pneumoniae in a Brazilian study (13), but it was similar to 12.8% among K. pneumonia found in a Colombian study (34) and much lower than 77% found in another
study performed in Colombia(17). That variation might be influenced by the extensive usage of these antibiotics in some hospitals, exerting selective pressure. 

*Serratia marcescens* has proved high resistance rates to most antimicrobials tested, and 91.7% of the isolates were MDR. Gentamicin and Trimethoprim-sulphamethoxazole had the higher susceptibility, while 87.5% were resistant to carbapenems. Those findings differ from the ones described in Latin America: more than 91% of all *S. marcescens* isolates were susceptible to carbapenems (36). This species is also capable of developing resistance to polymyxin B (37), which explains 95.2% of resistance to that antimicrobial, while all other Enterobacteria tested were susceptible.

A limitation in our study was the fact samples were collected in a secondary hospital, while most of the other studies were performed in tertiary hospitals. Also we analyzed samples collected in one year, and in this period it is not possible to analyze a tendency in drug resistance patterns at this hospital. Finally, we did not correlate these results with characteristics of the patients such as comorbidities and previous hospitalization, which may influence the prevalence of microorganisms found in this study.

**Conclusions**

These results corroborate the variation in microbiology and resistance patterns among different hospitals, and how important it is to know it to determine the best antimicrobials for empiric treatment in each service. This study provides relevant information to guide empiric therapy in our settings. It also draws attention to the fact there are important MDR strains susceptible only to a few broad spectrum antibiotics, leaving limited options for treatment. This scenario becomes more preoccupying considering the fact only a small number of antibiotics are in development.
References


Figure 1. Frequency of microorganisms isolated from blood cultures at a secondary Brazilian hospital in 2012 (n=1056).
Figure 2. Percentage of multidrug resistance among gram positive cocci isolated from blood cultures at a secondary Brazilian hospital in 2012.
Figure 3. Percentage of multidrug resistance, extended-spectrum beta-lactamases and carbapenemase in gram-negative bacilli isolated from blood cultures at a secondary Brazilian hospital in 2012
Table 1. Resistance of gram negative bacteria to antimicrobial agent

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Percentage resistance</th>
<th>A. baumannii</th>
<th>P. aeruginosa</th>
<th>K. pneumoniae</th>
<th>E. coli</th>
<th>Enterobacter sp.</th>
<th>S. marcescens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td></td>
<td>72.6</td>
<td>13.6</td>
<td>5.6</td>
<td>0</td>
<td>19.2</td>
<td>75</td>
</tr>
<tr>
<td>Ampicillin-sulbactum</td>
<td></td>
<td>40.5</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Cefepime</td>
<td></td>
<td>82.1</td>
<td>40.9</td>
<td>59.3</td>
<td>31.4</td>
<td>42.3</td>
<td>91.7</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td></td>
<td>85.7</td>
<td>45.5</td>
<td>59.3</td>
<td>31.4</td>
<td>61.5</td>
<td>83.3</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td></td>
<td>NT</td>
<td>NT</td>
<td>59.3</td>
<td>31.4</td>
<td>60.0</td>
<td>95.8</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>84.5</td>
<td>31.8</td>
<td>57.4</td>
<td>37.1</td>
<td>61.5</td>
<td>45.8</td>
</tr>
<tr>
<td>Ertapenem</td>
<td></td>
<td>NT</td>
<td>NT</td>
<td>16.7</td>
<td>0</td>
<td>70.6</td>
<td>87.5</td>
</tr>
<tr>
<td>Gentamicin</td>
<td></td>
<td>25</td>
<td>36.4</td>
<td>33.3</td>
<td>25.7</td>
<td>30.8</td>
<td>12.5</td>
</tr>
<tr>
<td>Imipenem</td>
<td></td>
<td>81.2</td>
<td>31.8</td>
<td>11.1</td>
<td>0</td>
<td>42.3</td>
<td>87.5</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td></td>
<td>79.7</td>
<td>NT</td>
<td>57.4</td>
<td>31.4</td>
<td>57.7</td>
<td>37.5</td>
</tr>
<tr>
<td>Meropenem</td>
<td></td>
<td>78.6</td>
<td>31.8</td>
<td>13.0</td>
<td>0</td>
<td>42.3</td>
<td>87.5</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td></td>
<td>81.2</td>
<td>13.6</td>
<td>38.9</td>
<td>2.9</td>
<td>53.9</td>
<td>87.5</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NT</td>
<td>0</td>
<td>95.2</td>
</tr>
<tr>
<td>Trimethoprim-sulphamethoxazole</td>
<td></td>
<td>NT</td>
<td>NT</td>
<td>57.4</td>
<td>44.1</td>
<td>57.7</td>
<td>16.7</td>
</tr>
<tr>
<td>Tobramycin</td>
<td></td>
<td>NT</td>
<td>NT</td>
<td>31.5</td>
<td>11.8</td>
<td>36.0</td>
<td>29.2</td>
</tr>
</tbody>
</table>

NT= not tested
Table 2. Resistance of gram positive cocci to antimicrobial agent

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>S. aureus</th>
<th>CoNS</th>
<th>E. faecalis</th>
<th>E. faecium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>100</td>
<td>99.2</td>
<td>0</td>
<td>75</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>46.3</td>
<td>67.1</td>
<td>45.9</td>
<td>66.7</td>
</tr>
<tr>
<td>Streptomycin*</td>
<td>NT</td>
<td>NT</td>
<td>56.8</td>
<td>33.3</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>14.8</td>
<td>48.8</td>
<td>29.7*</td>
<td>41.7*</td>
</tr>
<tr>
<td>Levofoxacin</td>
<td>44.8</td>
<td>65.7</td>
<td>43.2</td>
<td>66.7</td>
</tr>
<tr>
<td>Linezolid</td>
<td>0</td>
<td>0</td>
<td>NT</td>
<td>0</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>61.1</td>
<td>83.1</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Penicillin</td>
<td>100</td>
<td>99.5</td>
<td>NT</td>
<td>75</td>
</tr>
<tr>
<td>Trimethoprim-sulphamethoxazole</td>
<td>7.4</td>
<td>52.7</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>20.6</td>
<td>11.8</td>
<td>70.2</td>
<td>16.7</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*High level streptomycin, **High level gentamicin, CoNS, coagulase negative staphylococcus, NT, not tested
Table 3. Prevalence of microorganisms by hospital sector

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>UCI  n(%)</th>
<th>Wards n(%)</th>
<th>Emergency n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoNS</td>
<td>280 (53.7)</td>
<td>53(41.1)</td>
<td>229(56.4)</td>
</tr>
<tr>
<td>A. Baumannii</td>
<td>58 (11.1)</td>
<td>14(10.8)</td>
<td>12(2.9)</td>
</tr>
<tr>
<td>K. Pneumoniae</td>
<td>34 (6.5)</td>
<td>7(5.4)</td>
<td>11(2.7)</td>
</tr>
<tr>
<td>S. Aureus</td>
<td>27 (5.2)</td>
<td>25(19.4)</td>
<td>56(13.8)</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>20(3.8)</td>
<td>1(0.8)</td>
<td>5(1.2)</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>19(3.6)</td>
<td>9(7)</td>
<td>9(2.2)</td>
</tr>
<tr>
<td>S. Marcescens</td>
<td>19(3.6)</td>
<td>2(1.5)</td>
<td>5(1.2)</td>
</tr>
<tr>
<td>Total</td>
<td>521</td>
<td>129</td>
<td>406</td>
</tr>
</tbody>
</table>

UCI, intensive care unit; CoNS, coagulase negative staphylococcus
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The Brazilian Journal of Infectious Diseases

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Abstract


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