ORIGINAL ARTICLE

BACTERIOLOGICAL PROFILE ANALYSIS OF PATIENTS ADMITTED TO INTENSIVE CARE REGARDING MORBIDITY AND MORTALITY: A LONGITUDINAL STUDY

Danielle Mendonça Henrique¹; Andreza Moreno de Carvalho²; Flavia Giron Camerini³
Cintia Silva Fassarella¹; Robson Souza Leão⁵; Luana Ferreira de Almeida⁶

Highlights: (1) Prevention of Multidrug-Resistant Infections in Intensive Care Units.
(2) Monitoring the microbiological profile by intensive care nurses.
(3) Nursing practices for dealing with and combating antimicrobial resistance.

PRE-PROOF
(as accepted)

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¹ Universidade do Estado do Rio de Janeiro (UERJ). Rio de Janeiro/RJ, Brasil.
https://orcid.org/0000-0002-0656-1680
https://orcid.org/0000-0001-6212-4840
https://orcid.org/0000-0002-4330-953X
https://orcid.org/0000-0002-2946-7312
https://orcid.org/0000-0003-0636-1520
https://orcid.org/0000-0001-8433-4160
ABSTRACT

Objective: To analyze the bacteriological profile of patients admitted to an intensive care unit and its association with morbidity and mortality. Method: An observational, longitudinal study with a sample of 180 patients admitted to an intensive care unit. Data collection occurred through consultation of the clinical bacteriology laboratory database and electronic medical records between May and July 2022. The Kaplan-Meier method was used for survival analysis. Results: Klebsiella pneumoniae (46.38%) was the most prevalent microorganism, with resistance rates of 90.6% for cefepime, 84.4% for cefotaxime, 84.4% for ceftazidime, 84.4% for ciprofloxacin, and 84.4% for piperacillin + tazobactam. The study showed that with each day of hospitalization, the chance of the patient presenting a positive blood culture increased by 4.1% (p-value <0.001). Regarding mortality, 85 patients died, with 34 (62.96%) having a positive blood culture. The presence of microorganisms in blood cultures increased the risk of death by 2.5 times (p-value 0.006). Survival analysis showed that patients with positive blood cultures had a longer length of stay. Conclusion: Adequate knowledge of the microbiological profile and antimicrobial resistance by nurses is essential to guide the planning and implementation of care, managerial, and educational strategies for nursing and the entire multidisciplinary team.

Palavras-chave: microbiology; hospital-acquired infection; microbial drug resistance; intensive care units; patient safety.

INTRODUCTION

Healthcare-Associated Infections (HAIs) are considered adverse events, as they involve incidents resulting in patient harm arising from healthcare, with implications for healthcare quality and patient safety. Worldwide, millions of patients are affected by HAIs each year, leading to high mortality rates, increased length of hospital stay, and considerable financial losses for healthcare systems. Estimates suggest that 5 to 10% of patients utilizing hospital services acquire one or more infections\(^1\)-\(^2\).

Roughly 30% of patients admitted to Intensive Care Units (ICUs) are affected by at least one episode of Healthcare-Associated Infections (HAIs). The incidence rates of infections in
this setting vary from 44% to 88% in low- and middle-income countries, with high frequency associated with the need for procedures and the use of invasive devices for therapeutic maintenance\(^3\).

The vulnerability to bloodstream infection by microorganisms is highly relevant in the ICU, and its prevalence may be related to various factors. Among these, the following are highlighted: the flow of patients undergoing surgeries, prolonged hospital stays, diagnoses, and comorbidities, use of invasive devices, age, immunosuppression characteristic of critically ill patients, as well as empirical treatment with broad-spectrum antibiotics\(^4\).

The irrational use of broad-spectrum antimicrobials has become a challenge in the ICU, resulting in a higher prevalence of microorganisms resistant to such drugs. Antimicrobial resistance (AMR) is a global concern, defined by the World Health Organization (WHO) as the ability of microorganisms (bacteria, fungi, viruses, and parasites) to change in response to antimicrobial exposure and to resist these drugs, thereby losing their effectiveness. Each year, around 700,000 deaths are caused by infections derived from multidrug-resistant bacteria, and it is projected that by 2050, there will be approximately 10 million deaths per year\(^5\)\(^6\).

The main microorganisms involved in the occurrence of HAIs are gram-negative bacilli (GNB), which are particularly associated with increasing antimicrobial resistance. A study conducted with 143 patients with bloodstream infections (BSIs) caused by GNB showed that approximately 80.5% of the isolated microorganisms belonged to the Enterobacteriaceae group, with Escherichia coli and \(K.\) pneumoniae being the most frequent\(^7\).

Multidrug-resistant microorganisms have been classified by the WHO according to their critical priority of resistance and epidemiological significance. Considering that such bacteria, when present in infectious processes, increase the risk of mortality, they are urgently prioritized on the research agenda for the development of new antimicrobials. These bacteria include: \(Acinetobacter\) baumannii, \(K.\) pneumoniae and \(E.\) coli\(^8\).

Despite its significant impact, bloodstream infections have great potential for prevention. The analysis of blood culture results by nurses contributes to directing care, managerial, and educational strategies for preventing HAIs and antimicrobial resistance in the ICU, resulting in improved healthcare quality and patient safety.
The objective was to analyze the bacteriological profile of patients admitted to an intensive care unit and its association with morbidity and mortality.

**METHOD**

This is an observational, longitudinal, retrospective, and documentary study with a quantitative approach to data, guided by the recommendations of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE). It was conducted in an adult ICU located in a university hospital in the city of Rio de Janeiro.\(^9\)

For probabilistic sampling calculation, a simple random sample was chosen, measured using the Stat Calc Epi-Info calculator (version 3.5.1). The population considered was the patients admitted to the studied ICU in 2021 (265, according to hospital census data), with a sampling error of 5% and a confidence level of 95%. The sample was estimated at 157 patients and was extrapolated to 180.

For inclusion criteria, patients above 18 years old admitted to the ICU in the year 2021 were considered. Exclusion criteria included a length of stay of less than 72 hours, justified by the definition of Healthcare-Associated Infections (HAIs) by the Brazilian Health Regulatory Agency (ANVISA), which requires a minimum of two calendar days (starting from Day 3) for the occurrence of HAIs.\(^{11}\)

Data collection was conducted between May and July 2022. The data were collected from primary sources via electronic medical records and secondary sources via a spreadsheet from the clinical bacteriology laboratory containing records of blood culture results.

The study occurred in two stages as follows: the first stage involved data collection from the electronic medical records of patients admitted to the ICU in 2021 who met the inclusion criteria. In this stage, it was possible to trace those patients who had blood culture collection during their hospitalization. The second stage involved querying the clinical bacteriology laboratory database to confirm the results of patients who had positive blood cultures and identify the antimicrobial resistance profile.
It is worth noting that, considering the possibility of blood culture contamination leading to false-positive results and to avoid selection bias, contaminated blood cultures were identified as those where a single organism, such as skin-residing organisms - including coagulase-negative *Staphylococcus sp coagulase-negativos* (CoNS), *Cutibacterium acnes, Micrococcus spp, Streptococcus sp viridans group (VGS), Corynebacterium spp* or *Bacillus spp* – was isolated, and subsequent cultures were negative. These defined contaminated blood cultures were excluded from the study.

The data collection instrument included sociodemographic variables such as age, sex, length of stay, and outcome (discharge from the ICU or death). Regarding the bacteriological profile, variables were related to microorganisms and their classification based on the species isolated in blood cultures as gram-positive and gram-negative, as well as the presence of antimicrobial resistance.

The data were collected using a standardized form and organized in an electronic spreadsheet (Microsoft Excel®). For data analysis, the R software was utilized. Descriptive statistics were used to present patient characteristics, microorganism prevalence, and resistance profile using absolute and relative frequencies. Inferential statistics were employed to analyze the outcomes of death and length of stay in the ICU among patients with microorganisms isolated in blood cultures. For this purpose, Pearson's chi-squared test and Brunner-Munzel test were used. To analyze the survival time of the study patients, the Kaplan-Meier estimator was used. In the applied tests, the significance level was set at 95% (p <0.05).

This study was approved by the Research Ethics Committee involving Human Subjects, complying with all guidelines according to the Guidelines and Regulatory Norms for Research Involving Human Subjects, as outlined in Resolution of the National Health Council (CNS) No. 466/2012. under opinion number 3.960.

**RESULTS**

The presented results are based on the analysis of 180 patients admitted to the ICU. Regarding the sociodemographic profile, there was a balance between female (55%) and male (45%) patients, with the highest prevalence age group being 60 years or older, accounting for 83 (46.1%) patients. Regarding length of stay, the mean was 15 days (Standard Deviation [SD]:
23 days, with a maximum stay of 210 days and a minimum of 3 days). The outcomes for the analyzed patients included transfer to another department for 95 (52.78%) patients and death for 85 (47.22%) patients. It is noteworthy that the most prevalent cause of death in the unit was septic shock, accounting for 77.65%.

Among the 180 patients, 126 either did not have blood cultures collected or had negative results, while 54 tested positive. A total of 109 blood culture samples were traced, with 20 excluded due to suspected contamination, resulting in 89 valid samples. These were analyzed based on the prevalence of microorganisms and antimicrobial resistance profile.

Table 1 presents the association of numerical variables (age and length of stay) with the presence of microorganisms. It was evident that older patients were more likely to have positive blood cultures, with each year of age increasing the chance by 1.3%. Regarding the length of stay, statistical significance was identified (p-value <0.001), with each day of hospitalization increasing the chance of the patient having a positive blood culture by 4.1%.

Table 1 – Association of Age and Length of Stay with Positive Blood Cultures in Patients Admitted to the Intensive Care Unit. Rio de Janeiro, RJ, Brazil, 2022.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Positive Blood Cultures</th>
<th>n</th>
<th>Odds ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>No</td>
<td>126</td>
<td>1.013</td>
<td>0.155*</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length of Hospital Stay</td>
<td>No</td>
<td>126</td>
<td>1.041</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>54</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Wilcoxon-Mann-Whitney test **Brunner-Munzel test

Considering that patients in the intensive care unit are typically transferred to less complex units before hospital discharge, possible outcomes adopted include death and transfer from the ICU. Table 2 presents the association of hospital outcome with positive blood cultures. It is noteworthy that out of the 85 identified deaths, 34 (62.96%) had positive blood cultures. Regarding transfer, it was evident that out of the 95 transferred patients, 20 (37.04%) had positive blood cultures. The analyzed outcomes showed statistical significance (0.006), and the presence of microorganisms in blood cultures increased the risk of death by 2.5 times.

Table 2 – Association of Hospital Outcome with Positive Blood Cultures in Patients Admitted to the Intensive Care Unit. Rio de Janeiro, RJ, Brazil, 2022.
The comparative analysis of cumulative survival and length of stay using the Kaplan-Meier estimator showed that patients with positive blood cultures have a longer length of stay compared to patients without microbial growth.

Graph 1 - Analysis of the survival curve of patients admitted to the Intensive Care Unit. Rio de Janeiro, RJ, Brazil, 2022.

The microbiological specification through blood cultures revealed a predominance of gram-negative bacteria (GNB) 69 (79.31%), with *K. pneumoniae* (46.38%), *Proteus mirabilis* (10.15%), and *Serratia marcescens* (10.15%) being the most prevalent.

Table 3 presents the antimicrobial resistance rates of Gram-negative bacteria (GNB). *K. pneumoniae* showed higher resistance rates for fourth-generation cephalosporin - cefepime (90.6%), third-generation cephalosporins - cefotaxime (84.4%) and ceftazidime (84.4%), quinolones - ciprofloxacin (84.4%), beta-lactamase inhibitors - piperacillin + tazobactam (84.4%), and carbapenems - meropenem (75.0%) and imipenem (56.3%).
Table 3 – Antimicrobial resistance rates of Gram-negative bacteria isolated in blood cultures from patients admitted to the Intensive Care Unit. Rio de Janeiro, RJ, Brazil, 2022. (n=69)

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>Klebsiella pneumoniae (n=32)</th>
<th>Proteus mirabilis (n=7)</th>
<th>Serratia marcescens (n=5)</th>
<th>Acinetobacter sp (n=4)</th>
<th>Pseudomonas aeruginosa (n=5)</th>
<th>Providencia stuartii (n=3)</th>
<th>Enterobacter cloacae Complex (n=3)</th>
<th>Escherichia coli (n=2)</th>
<th>Enterobacter sp (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>7 (21.9)</td>
<td>4 (57.1)</td>
<td>2 (28.6)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>2 (50.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>15 (46.9)</td>
<td>4 (57.1)</td>
<td>NT</td>
<td>3 (60.0)</td>
<td>NT</td>
<td>2 (50.0)</td>
<td>1 (33.3)</td>
<td>3 (100)</td>
<td>NT</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>27 (84.4)</td>
<td>5 (71.4)</td>
<td>4 (57.1)</td>
<td>3 (60.0)</td>
<td>5 (100)</td>
<td>2 (50.0)</td>
<td>1 (33.3)</td>
<td>0</td>
<td>2 (100)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>27 (84.4)</td>
<td>5 (71.4)</td>
<td>3 (42.9)</td>
<td>1 (20.0)</td>
<td>NT</td>
<td>2 (50.0)</td>
<td>1 (33.3)</td>
<td>0</td>
<td>2 (100)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>27 (84.4)</td>
<td>5 (71.4)</td>
<td>1 (14.3)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>4 (100)</td>
<td>1 (33.3)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>2 (6.3)</td>
<td>0</td>
<td>0</td>
<td>5 (100)</td>
<td>2 (40.0)</td>
<td>4 (100)</td>
<td>1 (33.3)</td>
<td>0</td>
<td>NT</td>
</tr>
<tr>
<td>Imipenem</td>
<td>18 (56.3)</td>
<td>1 (14.3)</td>
<td>4 (57.1)</td>
<td>1 (20.0)</td>
<td>4 (80.0)</td>
<td>2 (50.0)</td>
<td>0</td>
<td>0</td>
<td>NT</td>
</tr>
<tr>
<td>Meropenem</td>
<td>24 (75.0)</td>
<td>5 (71.4)</td>
<td>4 (57.1)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>2 (50.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Piperacillin/Tazobactam</td>
<td>27 (84.4)</td>
<td>5 (71.4)</td>
<td>1 (14.3)</td>
<td>2 (40.0)</td>
<td>5 (100)</td>
<td>2 (50.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>6 (18.8)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Sulfamethoxazole/Trimethoprim</td>
<td>6 (18.8)</td>
<td>2 (28.6)</td>
<td>0</td>
<td>4 (80.0)</td>
<td>NT</td>
<td>2 (50.0)</td>
<td>1 (33.3)</td>
<td>NT</td>
<td>0</td>
</tr>
</tbody>
</table>

Legend: NT – Not Tested; Pipe + Tazo – Piperacillin + Tazobactam; Sulfa. + Trim. – Sulfamethoxazole + Trimethoprim
DISCUSSION

Understanding the bacteriological profile is essential to guide strategic actions aimed at reducing mortality in intensive care units, as the accuracy of etiological agents and antimicrobial resistance profile are crucial for the success of treatment and prevention of HAIs.

The sociodemographic data presented in this study revealed that in the age group above 60 years, the likelihood of having a positive blood culture increases during ICU admission. This finding is consistent with another study, where 45.71% of patients with positive blood cultures were aged over 60 years. It is noteworthy that mortality rates range from 16% to 49% for bloodstream infections in individuals aged 65-75 years and from 21% to 56% for elderly individuals aged 75 years or older.

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Regarding the length of hospital stay of the analyzed patients, this study found that patients with positive blood cultures had a longer length of stay, with an average of 27.41 days, compared to patients without this diagnosis, who had an average stay of 10.13 days. This result is consistent with another study, which showed an average hospital stay of 26.4 days for patients with Healthcare-Associated Infections (HAIs) and 6.4 days for patients without such infections.

Patients with positive blood cultures have prolonged length of stay and higher mortality rates. These data are consistent with findings from a study that identified that 66.7% of patients with HAIs died, while among patients without HAIs, only 14.3% had the same outcome. A retrospective cohort study compared the characteristics and outcomes of sepsis with positive and negative blood cultures, showing that patients with positive cultures had longer hospital...
stays (14 vs. 6 days, \( P < 0.001 \)) and higher hospital mortality (14.6% vs. 8.5%, \( P = 0.019 \)) than those with negative cultures\textsuperscript{17-18}.

Another study, which evaluated 106,586 admissions of critically ill patients between 2002 and 2017, employed a multivariable logistic regression model to analyze patients without blood culture collection, with negative blood cultures, and with positive blood cultures. A total of 78,568 blood cultures were evaluated. It identified that the 30-day hospital mortality without blood culture was 2.8% (95% CI 2.7; 2.9), negative blood culture 8.9% (95% CI 8.5; 9.3), and positive blood culture 16.7% (95% CI 15.5; 17.9). There was a significant interaction between positive blood culture and disease severity, OR 1.06 (95% CI 1.05; 1.08), and comorbidity, OR 1.09 (95% CI 1.09; 1.10)\textsuperscript{19}.

The most prevalent microorganism in the studied ICU was \textit{K. pneumoniae}, isolated in 32 (46.38%) blood cultures. This result is supported by the Patient Safety and Quality in Health Services Bulletin (2021), which reports that in 2020, \textit{K. pneumoniae} was the most isolated gram-negative bacteria in blood cultures in Brazil. Other studies conducted in national ICUs have also highlighted \textit{K. pneumoniae} as the most isolated etiological agent in blood cultures, reinforcing the findings of this research\textsuperscript{20-23}.

\textit{K. pneumoniae} is a gram-negative bacillus present in the human intestinal microbiota, characterized by the presence of a polysaccharide capsule and an iron uptake system. Mucoid phenotype and toxic lipopolysaccharide are important properties determining its virulence. Moreover, these microorganisms have the ability to persist for extended periods on the skin and in dry environments, such as hospital surfaces, which may explain their prevalence in intensive care units\textsuperscript{12}.

Bacterial resistance can occur either intrinsically, through bacterial evolution, or acquired, due to selective pressure from inappropriate antimicrobial use, leading to genetic mutations that can be transferred between species. It is important to highlight the high resistance of Acinetobacter sp., showing resistance rates ranging from 50% to 75% to the tested antibiotics. This data is consistent with other studies where Acinetobacter baumannii, a species of the Acinetobacter genus, exhibited some degree of resistance to all tested antimicrobials\textsuperscript{22-24}. 
When it comes to the characteristics of the *Acinetobacter* sp genus, it is a gram-negative microorganism commonly found on surfaces and carried on the hands of healthcare professionals. It is often associated with respiratory tract infections, especially in tracheostomized patients, as well as infections linked to venous catheters. This bacterium is also correlated with opportunistic infections in immunocompromised patients with severe illnesses, who undergo invasive procedures and broad-spectrum antibiotic therapy.  

A population-based study conducted in Israel revealed that nearly 50% of patients with bloodstream infections died within one year. The death of an affected patient led to an average loss of 13.2 potential years of life, and each event resulted in an average loss of 6.2 potential years of life. The annual mortality rate was 74.6 per 100,000 inhabitants, representing approximately 10% of all deaths in Israel and resulting in an estimated loss of 1,012.6 potential years of life per 100,000 inhabitants.  

The results highlight that bloodstream infection leads to a worse prognosis for critically ill patients. Therefore, multidisciplinary strategies involving physicians, pharmacists, microbiologists, intensivists, and nurses have been suggested as best practices for controlling HAI and AMR. Nurses can play a significant role in strategies to combat AMR, as they represent a large workforce in healthcare services and significantly influence care processes.

Among the key actions of intensive care nurses in managing, controlling, and preventing HAI and combating AMR is their active participation in early detection of infection cases, sample collection, antibiotic administration, treatment monitoring, adverse event monitoring, and accountability regarding antimicrobial treatment, aiming to optimize the use of agents. In this context, microbiological monitoring of critically ill patients can contribute to and guide prevention efforts, serving as a barrier to the dissemination of microorganisms.

It refers to the nursing team as the class that remains in direct contact with patients in the ICU for the longest period. Therefore, knowledge of the characteristics of microorganisms causing HAI by nursing professionals provides support for the implementation of targeted actions regarding unit management and professional practices to prevent the spread of these bacteria in healthcare facilities. Contact precautions are recommended for all infected patients, including the use of gloves, gowns, masks, goggles, and dedicated equipment (such as
oximeters, sphygmomanometers, thermometers), as well as the assignment of nursing staff exclusively for their care. Additionally, organizing training activities for professionals is crucial to ensure their understanding of the importance of maintaining infection control strategies\(^\text{30}\).

The study results highlighted the need for concern regarding particular species such as *Acinetobacter*, *K. pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, among others. Therefore, it is recommended to intensify cleaning and disinfection of the environment and equipment, use closed suction systems to prevent environmental contamination with aerosols containing the microorganism, and implement environmental surveillance culture as a means to reduce a potential source of environmental contamination, especially given the persistence of these microorganisms on surface\(^\text{30}\).

It is worth emphasizing the importance of directing the actions of the multidisciplinary team based on blood cultures, promoting rational antimicrobial use and management. It is relevant to highlight that during the COVID-19 pandemic, there was an increase in the misuse of antimicrobials, which led to an increase in multidrug-resistant infections. Therefore, measures implemented for patients, as well as the antimicrobial therapy used, should be guided by cultures and antibiograms to prevent the progression of antimicrobial resistance, which poses an imminent risk to global public health\(^\text{32}\).

**CONCLUSION**

The study revealed that the presence of multidrug-resistant infections, identified in positive blood cultures, is associated with increased mortality and length of stay in critically ill patients. Therefore, specific actions such as contact isolation, staffing for exclusive care, and rationalization of antimicrobial use can contribute to reducing this reality.

Adequate knowledge of microbiological profile and antimicrobial resistance by nurses is essential to guide the planning and implementation of care, managerial, and educational strategies for nursing and the entire multidisciplinary team.

The study's limitation lies in its single-unit setting and the use of secondary data sources. It is noteworthy that future studies should be conducted to foster actions and care to minimize the risk of adverse events and, consequently, contribute to improving the quality of
care by the multidisciplinary team.

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Danielle Mendonça Henrique: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing – review & editing.
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Andreza Moreno de Carvalho: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft.
Flavia Giron Camerini: Resources.
Cintia Silva Fassarella: Writing – review & editing.
Robson Souza Leão: Resources, Writing – review & editing.
Luana Ferreira de Almeida: Writing – review & editing.
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**Corresponding author:**
Danielle de Mendonça Henrique
Universidade do Estado do Rio de Janeiro (UERJ)
Blvd. 28 de Setembro, 157 - Vila Isabel, Rio de Janeiro/RJ, Brazil - CEP: 20551-030
E-mail: danimendh@gmail.com

Associate Editor: Dra. Christiane de Fátima Colet
Editor-in-Chief: Dra. Adriane Cristina Bernat Kolankiewicz

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