Microbiological mapping of clinical and environmental pharmacoresistance in intensive care units: a cross-sectional

Mapeamento microbiológico da farmacorresistência clínica e ambiental em unidades de terapia intensiva: estudo transversal

ABSTRACT

Objective: to carry out environmental and clinical microbiological mapping in Intensive Care Units. Method: a cross-sectional, descriptive and observational study, carried out in two Intensive Care Units of a university hospital. Samples of surfaces and hospital equipment from the patient unit were collected for microbiological analysis of microbial pharmacoresistance related to the results of microbiological cultures of patients' clinical samples. Results: the context of environmental contamination was analyzed in 14 patient units and their respective microbiological cultures. Of the total number of microorganisms isolated from clinical samples (25), 28.0% (7) were carbapenem-resistant Acinetobacter baumannii, 24.0% (6) carbapenem-resistant Pseudomonas aeruginosa, and 16.0% (4) polymyxin-resistant Klebsiella pneumoniae. Regarding patient unit environmental contamination, the most frequently contaminated surfaces were bed areas (64.3%), followed by (6) fixed surfaces of the unit (42.8%) and mobile and electronic equipment (28.6%). Conclusion: the presence of pharmacoresistant microorganisms was evidenced, the most frequent being carbapenem-resistance and agreement between species and phenotypic profile of clinical and environmental isolates.

Descriptors: Drug Resistance, Multiple, Bacterial; Cross Infection; Intensive Care Units; Housekeeping, Hospital; Microbiological Techniques.

RESUMO

Objetivo: realizar mapeamento microbiológico ambiental e clínico em Unidades de Terapia Intensiva. Método: estudo transversal, descritivo e observacional, realizado em duas Unidades de Terapia Intensiva de um hospital universitário. Foram coletadas amostras de superfícies e equipamentos hospitalares da unidade do paciente para análise microbiológica da farmacorresistência microbiana relacionadas aos resultados das culturas microbiológicas das amostras clínicas dos pacientes. Resultados: o contexto da contaminação ambiental foi analisado em 14 unidades de pacientes e suas respectivas culturas microbiológicas. Do total de microrganismos isolados das amostras clínicas (25), 28.0% (7) eram Acinetobacter baumannii resistente aos carbapenêmicos, 24,0% (6), Pseudomonas aeruginosa resistente aos carbapenêmicos, e 16,0% (4), Klebsiella pneumoniae resistente às polimixinas. Em relação à contaminação ambiental da unidade do paciente, as superfícies mais frequentemente contaminadas foram as áreas da cama (64,3%), seguidas de (6) superfícies fixas da unidade (42,8%) e equipamentos móveis e eletrônicos (28,6%). Conclusão: evidenciou-se a presença de microrganismos farmacoresistentes, sendo o mais frequente a resistência aos carbapenêmicos e a concordância entre as espécies e perfil fenotípico dos isolados clínicos e ambientais.

Descritores: Farmacorresistência Bacteriana Múltipla; Infecção Hospitalar; Unidades de Terapia Intensiva; Serviço Hospitalar de Limpeza; Técnicas Microbiológicas.
INTRODUCTION

Healthcare-associated infections (HAIs) have a multifactorial nature, being associated with factors intrinsic and extrinsic to patients. They can develop in different hospital environments, such as Intensive Care Units (ICUs). It is estimated that 30% of patients hospitalized in the ICU are at risk of developing at least one episode of HAI,\(^1\) due to the severity and hemodynamic instability of patients as well as the high level of technological complexity and multiple invasive procedures.\(^2\)

In relation to the most prevalent microorganisms in HAIs, there are the pathogenic groups called “ESKAPE”. The acronym ESKAPE defines the group of bacteria that encompass Gram-positive and Gram-negative species, composed of Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter species. Such pathogens have been gaining prominence and are present in the majority of hospital infections, due to morphological characteristics, such as pathogenicity factors as well as increasing resistance to multiple antimicrobial agents.\(^3\)

Microbial pharmacoresistance reduces the effectiveness of antimicrobial agents, increasing length of stay, in-hospital morbidity and mortality and increasing costs of care, resulting in one of the greatest challenges in public health today.\(^4\) Such resistance has become responsible for at least 700,000 annual deaths worldwide, estimated to reach 2.4 million people by 2050 due to therapeutic failures and a shortage of new antimicrobial agents.\(^5\)

The microorganisms that cause HAIs can be present in hospital furniture, equipment for individual and collective use, clothing and in the environment itself. Such fixed and mobile surfaces can function as sources or reservoirs of these microorganisms.\(^6\) The presence of these pathogens on surfaces exposes patients to the risk of infection by these agents.\(^7,8\)

Although there is a lot of research that deals with environmental contamination, there are still gaps in knowledge on this topic, considering that many of them do not specify the sensitivity profile of isolated microorganisms.\(^9-10\) They also do not consider the entire patient unit as a whole, limiting themselves to a few surfaces,\(^10-14\) as well as not investigating the etiological agents of infections and colonization of patients hospitalized in the unit under study.\(^15\)

The microbiological control of healthcare environments is the responsibility of the nursing team. In a systematic review, it was identified that nurses have great importance in carrying out microbiological surveillance and control actions, participating in
government hospital infection control programs, working in surveillance, monitoring and dissemination systems of HAI rates, providing incentives for campaigns aimed at adhering to standard precautionary measures, in addition to providing resources for continued education of health professionals.

Considering these gaps in knowledge, as well as the need to generate scientific evidence that supports nursing actions to control environmental contamination, this research proposed to carry out environmental and clinical microbiological mapping in intensive care patients.

**OBJECTIVE**

This research proposed to carry out environmental and clinical microbiological mapping in intensive care patients.

**METHOD**

**Study design and location**

This is a cross-sectional, descriptive and observational study, carried out in two ICUs of a tertiary level university hospital in the state of Paraná (PR), Brazil, a reference in high complexity for the Brazilian Health System (SUS - Sistema Único de Saúde).

The sectors investigated in this research were a General ICU (G-ICU), intended for treating adult patients, infected or colonized by multi-resistant microorganisms (MOMR) who require contact isolation, and an ICU intended for treating burn victims (B-ICU).

**Sample design and study period**

Patients hospitalized for a minimum period of 48 hours in the ICUs selected for the study and their respective units were included in the study. As for the exclusion criteria, the patient unit that previously went through the disinfection process before collection was considered.

Environmental microbiological samples from the patient unit in G-ICU and B-ICU were collected, respectively, on the 10th and 24th of February 2020, in a single period, by the team of trained researchers.

The sample of surfaces and equipment investigated considered the Brazilian Health Regulatory Agency (ANVISA - Agência Nacional de Vigilância Sanitária) standardization for patient unit composition, being selected based on the frequency of contact with professionals' hands and proximity to patients.

The patient unit components were divided into three groups:
- Group A: composed of fixed structures of the patient unit, including gas panel surfaces, IV pole, side table and granite countertop;
- Group B: composed of equipment from the patient unit, including surfaces of the mechanical ventilator, cardiac monitor and infusion pumps;
- Group C: composed of the surfaces of patients’ bed, with the exception of the mattress, which is disinfected during patients’ bath, according to the health institution’s routine.

Variables and data collection instrument

Data collection was carried out using a data collection instrument developed by the researchers containing patient demographic, clinical and microbiological data (name, length of stay and results of microbiological cultures referring to the stay in the investigated unit) and the patients’ respective unit (amount of equipment and inanimate surfaces investigated, results of environmental microbiological analyzes).

As for the microbiological data of patients occupying the beds studied, these were accessed in an electronic medical record, made available by Medview®. The positive results of microbial cultures carried out during patients’ stay in the studied sector until the date of collection of cultures from the environment were considered.

Procedure for collecting microbiological cultures from the environment

Cultures were collected by rubbing sterile swabs (Olen Kasvi®), moistened with sterile 0.9% saline solution over the entire surface to be collected, prioritizing the rubbing of the swab in areas of the surface with greater contact with the hands, such as buttons, handles, touch screen of devices, among others. After sample collection, the swabs were placed in Stuart medium and sent to the microbiology laboratory within a maximum period of four hours.

To identify the species and antimicrobial sensitivity profile, the swabs were inoculated in three tubes containing soy broth and trypticase (trypticase soy broth (TSB) - Kasvi®), the first containing 6.5% NaCl, the second containing cefotaxime (8µg/mL) and the third containing vancomycin. After incubation for eight hours at 35ºC, the vancomycin-resistant Enterococcus (VRE) broth was replicated on VRE agar (OXOID®, England), containing 6µg/mL of vancomycin, 6µg/mL of ciprofloxacin and colistin. TSB was replicated on MacConkey agar (Acumedia®) containing 8µg/mL cefotaxime, and NaCl broth was replicated on salted mannitol agar. The identification of microorganisms was carried out using a manual methodology recommended by Jorgensen. Sensitivity to antimicrobial agents was determined by the disk diffusion method following recommendations from the Clinical and Laboratory Standards Institute.
Dilution techniques were performed on Brain Heart Infusion (BHI) agar with vancomycin for Enterococcus spp. and Staphylococcus aureus. ATCC strains (standard strain – sensitive to antimicrobial agents) of S. aureus, E. faecalis, P. aeruginosa and K. pneumoniae were used as quality control, in addition to samples of Gram-negative bacilli resistant to third and fourth generation cephalosporins, monobactams and carbapenems (CR).

Multi-drug resistance was defined as bacterial non-susceptibility to one or more agents from three or more categories of antimicrobial agents, considering enterobacteria, Acinetobacter baumannii and Pseudomonas spp. resistant to 3rd or 4th generation cephalosporins or monobactams producing extended spectrum beta-lactamase (ESBL), CR and polymyxins (PR), oxacillin-resistant Staphylococcus aureus, VRE and multi-resistant (MR) microorganisms that did not fit into the aforementioned categories.\(^{19-21}\)

**Data Treatment and Analysis**

Data were tabulated in Excel\(^{\circ}\) and analyzed in simple and relative frequency. The results were presented in frequency graphs and descriptive tables.

Microbiological mapping was presented in a digital illustration of the patient unit created in SketchUp pro 2019 version 19.1.174. The images were randomized using Raylectron punch editor for professionals version 4.

**Ethical aspects**

This research is part of the objectives of the study “Investigação da contaminação ambiental em áreas críticas hospitalares e avaliação da efetividade da desinfecção”, approved by the Research Ethics Committee involving human beings at the institution, under CAAE (Certificado de Apresentação para Apreciação Ética - Certificate of Presentation for Ethical Consideration) 28169520.0.0000.5231.

**RESULTS**

The sample consisted of 14 patients who were hospitalized and their respective units, eight from the G-ICU and six from the B-ICU. The G-ICU has ten beds, however one was not occupied at the time of analysis and the other was excluded from the sample, as disinfection was carried out prior to swab collection. The mean period of hospitalization of patients in the G-ICU was 12 days, and in the B-ICU, 13 days, both varying from two to 22 days.

Of the total number of patients hospitalized at the time of the study, 13 (92.7%) had some infection or colonization with MOMR, eight (61.5%) in the G-ICU and five (38.5%)
in the B-ICU. The environmental cultures of these sectors identified the presence of MOMR in eight (87.5%) G-ICU units and in six (66.7%) B-ICU units, according to the distribution of clinical and environmental samples shown in Figure 1.

![Figure 1](image)

**Figure 1.** Mapping of the General Intensive Care Units (*G-ICU) and Burns Units (†B-ICU) according to the distribution of microorganisms isolated in clinical and environmental cultures according to the species and pharmacoresistance profile. Londrina, PR, Brazil, 2020

**Notes:** *G-ICU: General Intensive Care Unit; †B-ICU: Burns Intensive Care Unit; ‡MR: resistant to one or more agents from one or more categories of antimicrobial agents; §ESBL: extended-spectrum beta-lactamase; ¶PR: polymyxin-resistant.

Of the total patient units studied (14), in only one of them the patient was not infected or colonized by MOMR (B-ICU5). In two patient units, colonized or infected by MOMR, no environmental contamination was identified (G-ICU5 and B-ICU6).

Of the total number of microorganisms isolated from clinical samples (25), there was a predominance of *Acinetobacter baumannii* CR (7 - 28.0%), followed by *Pseudomonas aeruginosa* CR (6 - 24.0%) and *Klebsiella pneumoniae* PR (4 - 16.0%), as illustrated in Figure 2.
Figure 2. Distribution of isolated microorganisms and their respective antimicrobial resistance profile from patients’ clinical cultures in the General Intensive Care Unit (*G-ICU) and Burn Unit (†B-ICU). Londrina, PR, Brazil, 2020

Notes: *G-ICU: General Intensive Care Unit; †B-ICU: Burns Intensive Care Unit; ‡MR: resistant to one or more agents from one or more categories of antimicrobial agents; §ESBL: extended-spectrum beta-lactamase; ||CR: carbapenem-resistant; ¶PR: polymyxin-resistant.

Regarding the comparative analysis of MOMR isolated from clinical cultures and the environment, it was shown that 57.14% (8) of hospitalized patients showed concordance of the species and phenotypic profile of resistance between clinical and environmental samples. It was observed that 52.8% of microorganisms in clinical cultures were isolated in tracheal secretions, followed by tissue fragments (13.9%), urine (11.1%), peritoneal fluid (11.1%) and blood (11.1%) (Table 1).

Table 1. Relationship between clinical sample of patients hospitalized in the General Intensive Care Unit (*G-ICU) and Burn Unit (†B-ICU) and environmental sample from their respective beds. Londrina, PR, Brazil, 2020.
In relation to environmental contamination in both ICUs (Figure 3), the most frequently contaminated surface groups were C, related to the bed (9 - 64.3%), followed by A, referring to the unit’s fixed surfaces (6 - 42.8%). The group with the lowest frequency of contamination was B, composed of mobile and electronic equipment (4 - 28.6%). Regarding species and pharmacoresistance profile, A. baumannii CR was most common in all groups, followed by P. aeruginosa and Enterobacter spp. (Figure 3).
**Figure 3.** Distribution of multi-resistant microorganisms (*MOMR) isolated from Intensive Care Units according to surface groups, microbial species and pharmacoresistance profile. Londrina, PR, Brazil, 2020

**Notes:** *MOMR: multi-resistant microorganisms; †ESBL: extended-spectrum beta-lactamase; ‡CR: carbapenem-resistant; §PR: polymyxin-resistant.

In the distribution of incidence density of microorganisms from the ESKAPE group (Figure 4), isolated from clinical samples of patients from both units researched, during the year of this study, it is noteworthy that, in January and February, the period in which the samples for this study were collected, there was an increase in the incidence density of *A. baumannii* and *K. pneumoniae*, in line with the results obtained in the clinical and environmental samples of this research.

It is also observed that the incidence density of *A. baumannii* and *P. aeruginosa* remained high during the first quarter of 2020. Both were the most frequent in the environmental samples of this research. Furthermore, microorganisms that have not been found in the environment, such as *Enterococcus* spp. and *Staphylococcus aureus*, presented reduced incidence density rates between January and March.
Figure 4. Incidence density of microorganisms from the ESKAPE group in clinical cultures from study units from January to September 2020. Londrina, PR, 2020

DISCUSSION

High contamination was identified among the surfaces that make up the ICUs (78.57%). It was observed that A. baumannii CR was more frequent in both samples (clinical and environmental). It was found that 57.14% of patients showed concordance of species and phenotypic resistance profile between clinical and environmental samples.

Regarding the comparison analysis between clinical samples from hospitalized patients and the environmental sample, it was observed that the majority of patients in this research showed agreement between clinical samples and those isolated from the environment in relation to the bacterial species and the phenotypic profile of antimicrobial resistance. This finding indicates the possible relationship between environmental contamination and the processes of infection and colonization of patients as well as the potential risk of cross-contamination. However, a limitation of this study is the lack of analysis of isolated genotypes that could generate evidence of the clonal profile between clinical and environmental samples.
Research comparing environmental cultures from high-touch surfaces and clinical cultures from patients admitted to the ICU during an outbreak of A. baumannii CR resulted in genetic similarity greater than 95% of this resistant species between clinical and environmental samples, inferring that the clonal propagation of this microorganism has contributed to unit contagion.\(^8\)

One of the gaps in knowledge about environmental contamination is the restriction of analysis to certain surfaces, not obtaining a holistic view of patient unit contamination\(^22\). One of the first studies carried out, considering the hospitalized patient unit in the ICU, identified microorganisms on a wide variety of surfaces and concluded that other researchers’ efforts and the decontamination process must take into account the entire patient unit, not just objects and critical surfaces considered high risk,\(^23\) which was possible to observe in the current study, as the ICUs in their entirety were analyzed, including all the surfaces that make them up.

In the context of the patient unit, this research showed that bed surfaces presented a higher frequency of contamination by MOMR, corroborating the premise that surfaces with a higher degree of handling by professionals and patients can harbor a high number of pathogenic microorganisms.\(^24\)

Research carried out in a public hospital in northern Brazil, with the aim of assessing the occurrence of contamination by pathogenic microorganisms on inanimate surfaces in an Adult ICU, showed that the head of the bed was the second most frequently contaminated surface among the ten surfaces analyzed.\(^25\)

Research conducted in the ICU of a tertiary hospital collected samples from inanimate surfaces after carrying out terminal disinfection with free chlorine solution, resulting in the growth of MOMR in 52% (23/44) of samples analyzed. Methicillin-, vancomycin- and cephalosporin-resistant microorganisms were found. Most of these MOMR were in the immediate vicinity of patients, isolated in 33% of mattresses and curtains.\(^26\)

As for the MOMR found in this research, A. baumannii CR was most frequently found, followed by P. aeruginosa CR and Enterobacter spp. ESBL. Such microorganisms are commonly associated with the etiology of HAIs worldwide and make up a group of pathogens at high risk for multidrug-resistance called ESKAPE.\(^3\)

Research carried out comparing environmental samples from surfaces in an Adult ICU from a public hospital in northern Brazil and the respective clinical samples from patients hospitalized during the study period observed Acinetobacter baumannii as the
most prevalent strain, being present in clinical isolates from patients and on surfaces after the cleaning process.

Corroborating the findings of this research, in a study carried out in Rio Grande do Sul with the objective of determining MOMR in the ICU, 216 samples collected on ICU surfaces were analyzed (mechanical ventilators, oxygen valves, box of individual materials, individual cabinets, pumps infusion systems, cardiac monitors, patient lifting, stretcher rails, mattresses, sinks, liquid soap dispensers, dilution and nursing benches, keyboards, skirting creases, hemodialysis and X-ray equipment), being identified in such surfaces the prevalence of MOMR from the ESKAPE group, such as *Pseudomonas aeruginosa* (4.9%), *Klebsiella pneumoniae* (4.1%), *Acinetobacter baumanii* (2.5%) and *Escherichia coli* (0.82%).

It should be noted that, in this research, *Enterococcus* spp. was not found in environmental samples, diverging from research carried out in the same intensive care environment of this hospital in 2008, which found *Enterococcus faecium* VRE in 71% of the samples collected from the environment. This same research was characterized by an educational intervention with professionals, obtaining a significant reduction in environment and equipment contamination by VRE, when compared to the pre-intervention period (23.2%) to the post-intervention period (8.3%) (p = 0.001). This reduction has continued to this day, considering the results of this study.

Brazilian research, with the objective of assessing contamination by *Acinetobacter* spp. in an ICU, observed that all isolated A. baumannii strains were resistant to all antimicrobial agents tested in the research.

In the current study, microorganisms resistant to the CR and PR class of antimicrobial agents stand out in clinical and environmental findings. Such antimicrobial agents are considered, in many cases, the last options for treating infections caused by Gram-negative bacteria. It is known that CR were historically drugs of last resort for treating serious infections, however microorganisms developed resistance mechanisms with the production of enzymes that degrade CR. PR, used in the 1960s to treat serious infections, had high rates of nephrotoxicity and neurotoxicity associated with their prolonged use, and were then replaced by broad-spectrum cephalosporins and aminoglycosides, which had lower toxicity rates. However, with the emergence of resistance mechanisms to different classes of antimicrobial agents, PR were once again introduced into clinical practice and, in some cases, as the last therapeutic option still available.
Reinforcing this result, a study was carried out in ICUs of two public hospitals located in Brazil, one hospital in the Midwest region, consisting of 249 beds, including Neonatal and Adult ICU beds (clinical and surgical), and another in the North region, consisting of 98 beds, including Neonatal, Pediatric and Adult ICU beds (clinical and surgical). The authors found a prevalence of *Acinetobacter* spp. and *Pseudomonas* spp. in the first hospital, corroborating our results. However, in the second hospital analyzed, a prevalence of *Staphylococcus* spp. was found. The authors infer that this difference may have occurred due to the size of the hospitals and the cleaning/disinfection regimes adopted.

The results of this study showed the potential for environmental contamination in healthcare services by both pathogenic and antimicrobial-resistant microorganisms. In the current research, high contamination was observed among the surfaces that make up the ICUs (78.57%), confirming what is evidenced in other literature, such as research carried out in a university hospital in the hinterland of Vale do São Francisco, which identified 93.8% of the ICU equipment analyzed was contaminated by bacteria, highlighting the relevance of discussing this topic in HAI prevention.

This evidence can support nursing team actions both in cross-contamination prevention and in cleaning and disinfection protocol implementation in healthcare environments, given that these professionals work from the development of cleaning and disinfection protocols to supervision and audits of this process and execution of appropriate techniques to guarantee an environment free from microbial contamination and safe for human health.

**CONCLUSION**

The results of this research found the presence of drug-resistant microorganisms on ICU surfaces, particularly bed surfaces. The prevalence of resistance to the CR class in clinical and environmental isolates as well as the agreement between species and multidrug resistance phenotype profile of clinical and environmental isolates were evidenced.

These findings support the need for daily disinfection practices in healthcare environments as an important factor in controlling and preventing HAIs caused by MOMR.

Among the limitations of this study, the lack of molecular methods for genetic analysis of environmental and clinical isolates stands out, considering that the results of these analyzes could allow the identification of clones between species and the analysis
of agreement between clinical and environmental isolates. This limitation is justified by the high cost of molecular biology techniques.

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CONFLICT OF INTERESTS

Nothing to declare.

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