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# EVALUATION OF ANTIMICROBIAL RESISTANCE IN MICRO-ORGANISMS ISOLATED IN SURFACES OF THE INTENSIVE CARE UNIT OF A HOSPITAL IN TRES LAGOAS-MS, BRAZIL

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| ARTICLE INFO   | ABSTRACT  |  |  |  |  |
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| Article History:<br>Received 27 <sup>th</sup> December, 2020<br>Received in revised form<br>20 <sup>th</sup> January, 2021<br>Accepted 07 <sup>th</sup> February, 2021<br>Published online 17 <sup>th</sup> March, 2021<br>Key Words:<br>ICU, Microbiological profile,<br>Antimicrobial resistance and<br>Inanimate surfaces.<br>*Corresponding author: Alex M. Machado, | We investigated the microbiological profile and antimicrobial resistance of bacteria isolated in inanimate surfaces of the Intensive Care Unit (ICU) of a hospital in Tres Lagoas. Samples of the different surface of ten beds, equipment and instruments inside the ICU were collected. The samples (134) were seeded on blood agar, isolated and identified following a standard protocol. Were isolated 267 colonies, of which 198 were identified to the genus <i>Staphylococcus</i> (74.15%), where <i>Staphylococcus epidermidis</i> was the most prevalent (62.6%), followed by <i>Staphylococcus</i>   |  |  |  |  |
|  | aureus (31.3%), Staphylococcus saprophyticcus (6,1%) and others coccus: Enterococcus spp<br>(1.85%) and Micrococcus luteus (0.36%). Additionally, we identified 11 Gram negative bacilli<br>(4.06%), where the bacteria of Enterobacteracea family was the most prevalent mainly:<br>Pseudomonas aeruginosa (45.46%); Acinetobacter spp. (18.18%); Enterobacter spp., Klebsiella<br>pneumoniae, Providencia rettigeri, Serratia marcenses (9.09%). Additionally, 32 Gram positive<br>bacilli (11.81%) were isolated, mainly: Bacillus spp. (59.37%); Bacillus cereus (18.75%);<br>Bacillus circulans (9.38%); Bacillus coagulans (6.25%) and Bacillus subtilis (3.13%), Finally, 7<br>fungi (2.58%) were isolated, mainly: Penicillium spp. and Aspergillus spp. (42.86%) and Absidia<br>spp. (14.28%). We observed moderate resistance to antibacterials in all genders analyzed and |  |  |  |  |

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strains

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# **INTRODUCTION**

Health Care-Related Infections (HAIs) are a serious public health problem as they have a high rate of morbidity and mortality (Rener et al., 2013). Although the causes of nosocomial infection are related to the patient's susceptibility, to the diagnostic and therapeutic methods used, the main cause is still the conditions of asepsis and hygiene of the hospital environment (Sociedade Brasileira de Infectologia, 2001). These infections are also responsible for increasing the length of hospital stay, directly affecting hospital costs and also the indiscriminate use of antimicrobials, which favors the appearance of multi-resistant bacterial strains (Andrade et al., 2006). Antimicrobial resistance has become the main public health problem in the world, affecting all countries. It is an inevitable consequence of the indiscriminate use of antimicrobials, either by self-medication or by unnecessary prescription.

In some countries in Europe and North America, methicillin-resistant Staphylococcus aureus (MRSA), penicillin-susceptible Streptococcus pneumoniae (PNSSP), vancomycin-resistant Enterococcus (VRE) and broad-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL) it has appeared in increasing numbers in the community and in hospitals (Casadevall, 1996). Intensive Care Units (ICU) are considered epicenters of bacterial resistance, being the main source of outbreaks of multidrug-resistant bacteria, which cause a large number of nosocomial infections. In recent years, the increasing development of multi-resistant bacteria has caused a lot of concern in the scientific community, since resistance occurs to antibiotics in common use and even broad spectrum (Oliveira et al., 2008). Some healthcare-related infections are preventable and others are not. Preventable infections are those that can interfere with the transmission chain of microorganisms. The interruption of this chain can be carried out through simple and effective measures such as hand washing, the processing of articles and surfaces, the use of personal protective equipment, in the case of occupational risk and the observation of asepsis measures. Emergency physicians are often the first to prescribe antibiotics to patients. However, this does not justify the lack of concern with the selection of resistant bacteria. With the advent of permanent care and out-of-hospital health care institutions, there was an intersection between community infections by sensitive microorganisms and nosocomial infections by resistant bacteria (Akamine et al., 2010). It is true that in recent years, there has been a great achievement in relation to the fall in the number of deaths from infections, thanks to the advent of several drugs with a broad spectrum of action and better bioavailability. However, in the same period, the result of the combination of indiscriminate use of these drugs with the longest survival and invasive actions on patients generated a selection and induction of resistant bacteria (World Health Organization, 2014). Damasceno (2010) and Kramer, Schwebke, and Kampf (2006) point out that equipment and inanimate surfaces close to the patient, frequently touched by professionals, solutions and water can become contaminated and constitute a source of multi-resistant microorganisms. They report that there is still little research related to this subject, and that it is important to determine the epidemiological characteristics of microorganisms of clinical importance for each service, since the profile of infections varies between institutions, when present on surfaces, solutions, equipment and possible similarity with positive results from patient cultures.

Thus, knowledge about the prevalence of microorganisms and the profile of sensitivity and resistance to antimicrobials represent an important aid to health professionals and members of the Hospital Infection Control Commission so that they can identify the specific places that need a greater health strategy. prevention and control. In addition, the fact that the patients who are in these three units. in most cases, are in need of the use of antimicrobials, these results may contribute to the formulation of tactics aimed at the rational consumption of antibiotics, thus minimizing the appearance bacterial resistance and helping to discuss new treatment strategies that may interfere with the health and quality of life of patients and professionals. In this sense, the present study will add up in taking both clinical and governmental attitudes. Therefore, the objectives of this work were: To analyze the prevalence and resistance profile of Gram-positive and Gram-negative bacteria on inanimate surfaces of the Intensive Care Unit of a public/private hospital, in order to determine the resistance profile and sensitivity to antimicrobials specific to each isolated bacterial group.

# **METHODOLOGY**

A prospective study was carried out, based on the microbiological analysis of the inanimate surfaces of the Intensive Care Unit of a public/private Hospital, located in the municipality of Tres Lagoas – MS, Brazil. The research project was appreciated and authorized by the responsible (Hospital Director) and the researchers undertook to follow the rules that regulate research at the institution.

Samples collections and bacterial isolation: Samples of hospital surfaces present in the Intensive Care Unit of the hospital were collected in the period between January and July 2020. The inanimate surfaces collected were: stretcher, screen, respirator, multiparameter monitor, infusion pump, oxygen panel, sphygmomanometer, stethoscope, in addition to mice and computer keyboards present in this environment, as well as telephone, internal access doors and air conditioning (Table 1). The size of the samples (number of beds) to be collected in this environment was determined with the help of the free program with open code for epidemiological statistics - OpenEpi (http://www.openepi.com/Menu/OE\_ Menu.htm). The sample size was calculated based on a 20% frequency of the total sample of each surface of each area of the hospital, in which, is estimated that 20% of infections related to health care are attributed to inanimate surfaces. The confidence interval used for the calculation was 95%. Thus, were selected 10 ICU beds for evaluated. The recovery of surface samples was performed according to standardized protocols.

Following this study, the collection consisted of using a sterile cotton swab moistened with sterile 0.9% NaCl solution and subsequently placed in a sterile tube containing 1ml of 0.9% NaCl. The collections were carried out in the morning, after cleaning the wards by the hospital cleaning teams, as a way of evaluating the efficacy of sanitation. The tube swab with saline solution was applied to the analyzed surface in a zig and zag pattern, rotating and pressing it, repeating the pattern at an angle of 90° to the first friction and returning it to the tube with saline solution. The swab tubes were sent to the Laboratory of Microbiology of the Federal University of Mato Grosso do Sul. An aliquot (100 µL) of the solution formed was seeded with a platinum loop on Blood agar medium (non-selective rich medium) and MacConkey agar (selective for gram negative bacteria) prepared according to the manufacturer's specifications. All samples were sown in triplicate in the culture media. The plates were incubated at  $35 \pm 2$  ° C for 24 hours in a bacteriological incubator. After growth, colony morphology analyzes were performed, as well as presumptive staining tests and determination of large bacterial groups. The isolates were transferred to Brain Heart Infusion - BHI broth to assemble a bank of microorganisms and stored at room temperature until the completion of all collections. In plates with growth of more than one type of colony, isolation was carried out and only afterwards was the transfer of each of them to the BHI broth. At the end of the collections, the isolates were re-raised in BHI broth in order to make bacteria with slow metabolism viable, before being transferred to solid medium. From the growth in BHI broth, isolation was performed on Müeller-Hinton agar to certify the growth of the isolated bacterium. The isolated colonies were then subjected to identification by phenotypic tests using tests and panels for identification according to the identified gender.

Identification of bacteria (Gram Positive and Gram Negative): The colonies of staphylococci are generally larger, convex, with a color ranging from white to porcelain to yellow, with hemolysis or not. Streptococcal colonies tend to be smaller (punctate), and with halos of total or partial hemolysis (beta and alpha hemolysis). The differentiation between streptococci and staphylococci is certainly due to the catalase test. After differentiating Staphylococcus spp. of Enterococcus spp. using the catalase test, a coagulase test was performed to differentiate between Staphylococcus aureus (positive coagulase) and for those who gave negative coagulase, the Novobiocin sensitivity test was performed. The identification of Enterobacteria can be carried out in several ways, the main ones being biochemical tests. In this work, we used the lactose tests, visualized by changing the color of the MacConkey medium, oxidase and the Rugai and Araújo method Modified by Pessoa and Silva, which consists of a medium used for the presumptive identification of enterobacteria, vibers and aeromones.

Evaluation of the profile of sensitivity to antimicrobials: The sensitivity profile to antimicrobials was evaluated by the diskdiffusion technique as described in document M02-A11, from the Clinical Laboratory Standard Institute - CLSI. For each fresh sample, grown on Mueller-Hinton agar, a bacterial suspension was prepared in 4 mL of saline solution with turbidity comparable to the 0.5 McFarland scale. After homogenization, the suspension was seeded on a Müeller-Hinton Agar plate using a sterile swab in 8 different directions. The antibiotic discs were applied to the plate 50 mm apart. The plates sown with the discs were incubated at  $35 \pm 2$  ° C for 24 hours. The selection of antimicrobial disks, specific to each bacterial group, as well as the interpretative patterns of the inhibition halos were performed following a standardized protocol. After identifying the sensitivity profile of the bacteria, comparisons were made between the different areas analyzed, in order to understand the profile of sensitivity in the different bacterial species found in the different areas of the hospital.

# RESULTS

We obtained 134 samples of which 267 colonies were isolated, where 198 are of the genus *Staphylococcus* (74.15%), followed by *Bacillus* 

(11.81%) and Enterobacteria (4.06%). Among the most prevalent species of the genus *Staphylococcus*, the following species were identified: *Staphylococcus epidermidis* (62.6%), followed by *Staphylococcus aureus* (31.3%) and *Staphylococcus saprophyticus* (6.1%). Among Gram-positive cocci, *Micrococcus luteus* (0.36%) and *Enterococcus* spp. (1.85%) were also isolated. Gram positive shards showed moderate resistance to the different tested antibiotics (Average 26.30%) where the antibiotics for which there was greater resistance were Erythromycin (53.78%), Ceftazidime (38.68%) and Clindamycin (31, 13%). The antibiogram detected the presence of MRSA (*Staphylococcus aureus* Methicillin Resistant) in 35 (56.45%) strains of *S. aureus*. The highest prevalence was found in infusion pumps, respirator, screen and computer keyboards (Table 2)

Acinetobacter baumanni (18.18%), Enterobacter spp. (9.09%), Klebsiella pneumoniae (9.09%), Providencia rettigeri (9.09%) and Serratia marcenses (9.09%). The assessment of sensitivity and antibiotic resistance of these isolates showed, again, a moderate resistance (36.11%) where the antibiotics analyzed with the highest resistance rates went to Aztreonam (62.50%), Amoxicillin (50.0%), Ceftazidime (50.0%) and Cefepime (50.0%). Among the enterobacteria, no multiresistant strain was observed. In parallel, the antibiotics with the highest sensitivity rates were: Amikacin (100%), Ciprofloxacin (87.50%) and Imipenem (87.50%) (Table 3). Finally, among the isolated microorganisms, 32 were Gram-positive bacilli (11.98%), with the species Bacillus cereus (18.75%), Bacillus circulans (9.38%), Bacillus coagulans (6.25%) being identified and

| Table 1. Collection points for equipment/materials and furniture in the ICU. |
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|--|

| Equipment/materials                 | Collection points   |
|-------------------------------------|---|
| Mechanical respirator (When in use) | Panel (command buttons)   |
| Infusion pumps                      | Panel (command buttons)   |
| Multi-parameter cardiac monitor     | Panel (command buttons)   |
| Apples                              | Central region of the Upper, Middle and lower rails on the right side and headrest adjustment |
|                                     | handles located at the bottom of the stretcher.   |
| Stethoscope                         | Diaphragm (patient contact face)  |
| Sphygmomanometer                    | Rigid structure fixed close to the patient  |
| Oxygen panel                        | Oxigen cylinder conector  |
| Internal exit door handles          | External and central regions  |
| Telephone                           | Device hook and bottons   |
| Computers                           | Keyboard and Mouse  |
| Air conditioner                     | Air outlet pallets  |

#### Table 2. Profile of sensitivity and antimicrobial resistance of Gram-positive cocci isolated from inanimate surfaces of the ICU

| Antibiotic    |    | Absolute | Percentage | Antibiotic   |   | Absolute | Percentage |
|---------------|----|----------|------------|--------------|---|----------|------------|
| Amoxicillin   | S  | 53       | 50,00%     | Clindamycin  | S | 73       | 68,87%     |
|               | R  | 15       | 14,15%     |              | R | 33       | 31,13%     |
|               | UR | 38       | 35,85%     | Erythromycin | S | 49       | 46,22%     |
| Ceftazidime   | S  | 65       | 61,32%     |              | R | 57       | 53,78%     |
|               | R  | 41       | 38,68%     | Streptomycin | S | 100      | 94,34%     |
| Ceftriaxone   | S  | 79       | 74,53%     |              | R | 6        | 5,66%      |
|               | R  | 27       | 25,47%     | Sulfazotrim  | S | 82       | 77,36%     |
| Ciprofloxacin | S  | 86       | 81,13%     |              | R | 24       | 22,64%     |
| *             | R  | 20       | 18,87%     | Total (848)  | S | 587      | 69,22%     |
|               |    |          |            | . ,          | R | 223      | 26,30%     |

S: Sensitive; R: Resistant; UR: Unrealized. Total: UR. 38 (4,48%).

Table 3. Profile of sensitivity and resistance to antimicrobials of Gram-negative bacilli isolated from inanimate surfaces of the ICU

| Antibiotic  |   | Absolute | Percentage | Antibiotic    |   | Absolute | Percentage |
|-------------|---|----------|------------|---------------|---|----------|------------|
| Amoxicillin | S | 4        | 50,0%      | Cefepime      | S | 4        | 50,0%      |
|             | R | 4        | 50,0%      | •             | R | 4        | 50,0%      |
| Amikacin    | S | 8        | 100%       | Ciprofloxacin | S | 7        | 87,50%     |
|             | R | 0        | 0%         | *             | R | 1        | 12,50%     |
| Aztreonam   | S | 3        | 37,50%     | Imipenem      | S | 7        | 87,50%     |
|             | R | 5        | 62,50%     | -             | R | 1        | 12,50%     |
| Ceftazidime | S | 4        | 50,0%      | Sulfazotrim   | S | 4        | 50,0%      |
|             | R | 4        | 50,0%      |               | R | 4        | 50,0%      |
| Ceftriaxone | S | 5        | 62,50%     | Total (72)    | S | 46       | 63,89%     |
|             | R | 3        | 37,50%     |               | R | 26       | 36,11%     |

S: Sensitive; R: Resistant

Table 4. Profile of sensitivity and resistance to antimicrobials of Gram-positive bacilli isolated from inanimate surfaces of the ICU

| Antibiotic    |   | Absolute | Percentage | Antibiotic   |   | Absolute | Percentage |
|---------------|---|----------|------------|--------------|---|----------|------------|
| Ampicillin    | S | 17       | 56,67%     | Erythromycin | S | 25       | 83,33%     |
| •             | R | 13       | 43,33%     |              | R | 5        | 16,67%     |
| Azithromycin  | S | 24       | 80,0%      | Imipenem     | S | 27       | 90,0%      |
| -             | R | 6        | 20,0%      |              | R | 3        | 10,0%      |
| Ciprofloxacin | S | 28       | 93,33%     | Penicillin   | S | 20       | 66,67%     |
|               | R | 2        | 6,67%      |              | R | 10       | 33,33%     |
| Clindamycin   | S | 18       | 60,0%      | Total (210)  | S | 159      | 75,71%     |
|               | R | 12       | 40,0%      |              | R | 51       | 24,29%     |

S: Sensitive; R: Resistant

Additionally, we identified 11 Gram-negative bacilli (4.11%), belonging to the *Enterobacteriacea* family. Among the species isolated were found: *Pseudomonas aeruginosa* (45.48%),

*Bacillus subtilis* (3.13%) and *Bacillus* spp. (59.37%), therefore, it is not possible to identify the species in more than half of the analyzed bacilli.

The analysis of antibiograms showed that the highest rates of resistance were for antibiotics: ampicillin (43.33%), clindamycin (40.00%) and penicillin (33.33%). In addition, these Gram-positive bacilli showed high sensitivity to the following antibiotics: Ciprofloxacin (93.33%), Imipenem (90.0%) and Azithromycin (80.0%) (Table 4).

# DISCUSSION

Intensive care units (ICUs) are of essential importance to provide life support for severe organ failures and intensive monitoring capable of allowing early identification and the necessary treatment of serious clinical complications in critically ill patients, acting decisively when there is instability of organs and functional systems with risk of death (Santos et al., 2016). Among the Gram-positive cocci, the most found was S. epidermidis with 62.69% and S. aureus with 30.84%. These microorganisms, in particular Staphylococcus aureus and Staphylococcus epidermidis are among the most important related to Health Care Related Infections (HAI) in the ICU. The environment can act as a reservoir for MRSA, which consequently can contaminate a range of hospital equipment and survive for a long period of time, with the health team being the main carrier of these microorganisms among inpatients (Ferreira et al., 2011). These bacteria are colonizing species of the skin and nose, being more commonly inoculated during invasive procedures or distributed by the health team itself, mainly by hands, but also by fomites, and this situation is aggravated by the emergence of endemic multidrugresistant strains in the hospital environment (Moura, 2011). In a study carried out in a hospital in Porto Alegre, it was observed that the coats of health professionals as those responsible for the dissemination of both S. aureus (MRSA). S. epidermidis may be responsible for endocarditis and septicemia associated with catheters and prosthetic implants in immunodepressed patients (Brasil, 2010; Moura, 2011). Studies carried out in Brazil demonstrate the prevalence of 40 to 60% of MRSA in isolated samples from hospitalized patients. In these microorganisms, resistance is the result of the chromosomal mecA gene, which produces a new penicillinbinding protein (PBP) with low affinity for beta-lactams (Souza Júnior et al., 2009). Ferreira and collaborators (2011) carried out a microbiological study on inanimate surfaces of the ICU of a teaching hospital in Tres Coroas - MG, totaling 63 samples and of the 48 samples positive for S. aureus, 29 (60.4%) were resistant to methicillin. In addition to MRSA, other strains of the Staphylococcus genus have been isolated from hospital infections, which demonstrates that this group has developed resistance to several antimicrobials, including methicillin (SADER et al, 2006). In this study, of the antibiotics analyzed, the highest resistance rates were for erythromycin (53.78%), ceftazidime (38.68%) and clindamycin (31.13%).

The hands of patients and/or professionals act as one of the main sources of transmission of microorganisms. A 1997 survey by Boyce et al found that 42% of nurses contaminated their gloves with MRSA while performing procedures that involved touching objects in the rooms of patients with MRSA, even though this did not require direct contact with the patient. The Staphylococcus genus is commonly associated with skin infections and/or wounds and its control is essential in the hospital environment. The members of the Enterobacteriaceae family are gram-negative microorganisms found in nature and isolated from biological material, which colonize the gastrointestinal tract of humans as an integral part of the normal microbiota of these organs, making it a potential reservoir for these pathogens. Carbapenem-resistant enterobacteria (CRE) have emerged as an important cause of nosocomial infections worldwide and are characterized by rapid and progressive spread (Lavagnoli et al., 2017). Currently, they represent an important public health problem worldwide, since infections due to CRE have a high mortality rate, with limited therapeutic options. In recent years, the incidence of nosocomial infection associated with resistant microorganisms has increased worldwide. This resistance to antibiotics develops as a natural consequence of the bacterial population's ability to adapt. The

indiscriminate use of antibiotics increases the selective pressure and also the opportunity for the bacteria to be exposed to them. This opportunity facilitates the acquisition of resistance mechanisms, becoming the main public health problem in the world, affecting all countries. In general, these bacteria showed high rates of resistance to antibiotics, including resistance to other classes and cross-resistance. In addition, the spread of these multi-resistant bacteria occurs frequently due to the peculiar characteristics of the ICUs, such as: high frequency of contact between the health professional and patients, restricted unit, greater possibility of cross-transmission of the pathogen, high selective pressure for antibiotics of broad spectrum, greater likelihood of environmental contamination, use of drugs that interfere with the natural chemical barrier or alter the immune response, and use of tubes and catheters that hinder the physiological elimination of microorganisms (Abreu et al., 2011). The genus Bacillus comprises about fifty species of facultative

anaerobic bacilli that can present sporulated form. The vegetative forms are broad, straight and can be large, isolated or in chains. They form endospores and their location helps in their classification. They can be cylindrical, oval, round and sometimes bean-shaped. The endospores can be located centrally, subterminally or terminally, and may or may not dilate the mother cell.

All bacilli show mobility, except B. anthracis and B. mycoides, and the vast majority of them are catalase positive. In anaerobiosis or carbon dioxide (CO2) and in the presence of bicarbonate ions (HCO3-), B. anthracis, B. licheniformis and B. megaterium have a polypeptide capsule (Brasil, 2004). These microorganisms are found mainly in soil, water and in organic animal and vegetable matter under varying conditions of temperature, humidity and pH. Bacillus cereus is often related to necrosis or soft tissue gangrene, bacteremia and sepsis, pulmonary infectious conditions, endocarditis, meningitis, osteomyelitis and endophthalmitis, in addition to food poisoning that can present itself in two different ways: the first characterized by nausea and vomiting in 1 to 5 hours after eating contaminated food, in which case, the most common, rice; or even, it can be characterized by diarrhea and abdominal pain in 8 to 16 hours after eating a variety of foods, including meats, vegetables, milk, sauces, pasta, sweets and cakes. Bacillus circulans is responsible for infections in soft tissues, abscesses, bacteremia and sepsis. Bacillus subtilis is a common cause of food poisoning, and more rarely, bacteremia, sepsis, endocarditis and respiratory infections (Brasil, 2004). In view of this scenario, exposed above, we verified the need to implement control actions, through the improvement of cleaning in the environment, since these microorganisms are commonly found in dust. In addition, the constant monitoring of the presence of microorganisms in these sectors, as well as their sensitivity and resistance profile can provide data and information for a search for better ways to reduce the presence of these microorganisms, as well as to control possible cases of nosocomial infections.

# CONCLUSION

Microbiological analysis of inanimate surfaces can be useful in epidemiological investigations that suggest the environment or surfaces as possible reservoirs or sources of transmission of nosocomial diseases. Therefore, inanimate surfaces close to patients, as well as equipment that may have direct contact with them, are potential contaminants with epidemiologically important microorganisms and should be cleaned regularly, according to the hospital routine. The results found in this study represent a health risk for both the patient and the professionals due to the high incidence of environmental contamination. This may reflect the low adherence to hand hygiene and compromised environmental hygiene measures. Thus, educational actions and preventive interventions must be carried out to reduce contamination in the analyzed sector. The Hospital Infection Commission (CCIH) should insist on and promote training, re-education and incentive programs for good practices, including the correct technique for hand washing and periodic disinfection of equipment, as well as improving the cleanliness of the environment and more control. rigorous in relation to the

indiscriminate use of antibiotics in an empirical way. Only with targeted actions will it be possible to decrease the spread of microorganisms and the resistance of bacteria. Monitoring is essential to control pathogenic and multidrug-resistant strains. Therefore, additional studies are needed where future investigations of the clinical significance of contamination of the hospital environment and more effective cleaning methods can be carried out for better conclusions.

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