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Faucets and keyboards as a major reservoir of Gram-negative bacilli in a regional hospital in Northeast Brazil

Torneiras e teclados como importantes reservatórios de bacilos Gram-negativos em um hospital regional do Nordeste do Brasil

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ABSTRACT

Infections related to healthcare assistance represent a major public health problem worldwide, and the main etiological agents associated with these infections are microorganisms also found on inanimate hospital surfaces. Therefore, understanding the dynamics of bacterial contamination of hospital surfaces is essential for professionals working in these services. **Objective:** To evaluate the contamination of inanimate surfaces by Gram-negative bacilli (GNB) in a Regional Hospital in Northeast Brazil. **Methods**: This is an observational and cross-sectional study. Samples were collected from different hospital wards using a sterile swab soaked in saline solution. They were then plated on MacConkey agar and incubated at 36°C for 48 hours. The bacterial isolates were counted and identified using the Gram staining method, TSI agar, and oxidase test. **Results:** Out of the 150 samples collected, 71 showed no microbiological growth, 11 showed growth of filamentous fungi, and 66 showed growth of Colony Forming Units (CFU). A total of 132 morphologically distinct colonies were identified, with 55 identified as yeasts and 77 as GNB. In the evaluation of hospital wards, the Adult Emergency Room and the Orthopedic Surgical Clinic showed the highest growth of GNB. Regarding the surfaces studied, the most contaminated were faucets, computer keyboards, mattresses, and beds. **Conclusions:** Hospital areas with higher human traffic are the most contaminated, particularly on frequently handled objects. Therefore, it is necessary to focus on disinfecting these surfaces to break the transmission chain of these etiological agents.

Keywords: Gram-negative bacteria; equipment and supplies; hospital; disinfection; infection control.

RESUMO

As infecções relacionadas à assistência à saúde representam um grande problema de saúde pública em todo o mundo, e os principais agentes etiológicos associados a essas infecções são microrganismos também encontrados em superfícies hospitalares inanimadas. Desse modo, compreender a dinâmica de contaminação bacteriana das superfícies hospitalares é essencial para os profissionais que atuam nesses serviços. **Objetivo:** avaliar a contaminação de superfícies inanimadas por bacilos Gram-negativos (BGN) em um Hospital Regional do Nordeste do Brasil. **Métodos:** trata-se de um estudo observacional e transversal. As amostras foram coletadas em diferentes alas do hospital através de *swab* estéril embebido em solução salina. Foram semeadas em ágar MacConkey e incubadas a 36° C por 48 horas. Os isolados bacterianos foram contados e identificados pelo método de coloração de Gram, ágar TSI e teste de oxidase. **Resultados:** das 150 amostras coletadas, 71 não apresentaram crescimento microbiológico, 11 apresentaram crescimento de fungos filamentosos e 66 apresentaram crescimento de Unidades Formadoras de Colônias (UFC). Foram identificadas 132 colônias morfologicamente distintas, sendo 55 identificadas como leveduras e 77 como BGN. Na avaliação das alas hospitalares, o Pronto Socorro Adulto e a Clínica

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Ortopédica apresentaram maior crescimento de BGN. Quanto às superfícies estudadas, as que apresentaram maior contaminação foram torneiras, teclados de computador, colchões e camas. **Conclusões:** as áreas hospitalares com maior fluxo de pessoas são as mais contaminadas; a contaminação ocorre, sobretudo, em objetos com alta frequência de manipulação. Nesse sentido, é necessário focar na desinfecção dessas superfícies para quebrar a cadeia de transmissão desses agentes etiológicos.
Palavras-chave: bactérias Gram-negativas; equipamentos e provisões hospitalares; desinfecção; controle de infecções.

INTRODUCTION

The hospital environment is one of the main reservoirs of nosocomial bacteria, microorganisms capable of causing Healthcare-Associated Infections (HAIs). These infections are presented in hospitalized patients under medical care in a hospital or other healthcare facility and were absent at admission. HAIs pose a global concern for public health, representing a significant cause of morbidity and mortality in modern intensive care medicine.[1-4]

Among the modes of transmission, it is assumed that direct transmission by medical and nursing teams is the most common mode of exogenous transmission through hands. However, equipment and inanimate surfaces are among the fomites with the highest potential for transmitting nosocomial bacteria. Thus, the contamination of hospital surfaces can be mentioned, although cross-contamination by hand is possibly the greatest risk.[5]

According to studies, chairs, sphygmomanometers, and patient beds are some of the most contaminated inanimate objects. However, with the addition of computers in the hospital environment, microbial contamination of computer keyboards and inanimate surfaces of electronic equipment in Intensive Care Units (ICUs) may play a significant role in acquired colonization in this environment and the spread of nosocomial infections, as they come into contact with providers hands more frequently.[4,5]

Regarding microorganisms, numerous types of bacterial species can be identified contaminating inanimate surfaces, including Gram-negative bacilli (GNBs) such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter* spp., among others. Given their opportunistic action, these microorganisms represent some of the major pathogens causing nosocomial infections, affecting mainly immunocompromised hospitalized individuals and those with serious underlying diseases. This capability is attributed to intrinsic structures in their morphology, such as the production of adhesion molecules and the ability to form biofilms, gaining clinical importance due to their high capacity for acquiring antimicrobial resistance mechanisms.[6-8]

In this context, contaminated hospital surfaces constitute environments that require heavy, complex resources, and costly procedures to control these microorganisms and ensure patient safety.[3] Therefore, assessing the dynamics of bacterial contamination of inanimate surfaces, as well as identifying the microbial load present in them, is essential for understanding the transmission risks to patients and the medical team. Additionally, it is a strong tool for implementing stricter cleaning measures to prevent the spread of these microorganisms.

Therefore, this study aimed to identify the main inanimate surfaces that can act as reservoirs for GNBs in a Regional Hospital in Northeast Brazil.

METHODS

Sample collection

This observational and cross-sectional study was conducted at Tarcísio Maia Regional Hospital in the city of Mossoró, Rio Grande do Norte, Brazil.

The study was conducted from March to April 2019. A total of 150 collections were performed on different surfaces in the hospital environment with patient flow, without prior notice in the morning shift. The following hospital wards were included: Orthopedic Surgical Clinic (OSC), General Surgical Clinic (GSC), Medical Clinic (MC), Pediatric Clinic (PC), Infected Patients Unit (IPU), Intensive Care Unit (ICU), Pediatric Emergency Room (PER), Adult Emergency Room (AER), and Surgical Center (SC). Those responsible for the locations where the collections were made agreed to participate in the study.

In OSC, GSC, MC, PC, IPU, and PER, samples were collected from 15 surfaces, while in ICU, AER, and SC, samples were collected from 20 surfaces. The surfaces selected in common across all nine hospital wards were: nursing station countertops, medication preparation countertops, medication boxes, bed rails, including long and short-stay beds (X-ray and surgery), emergency carts, long and short-stay mattresses, light switches, door handles, gas panels, walls, clipboards, IV poles, sink faucets, and telephone or computer keyboards. Samples were collected from accompanying chairs in all wards, except the surgical center.

The additional surfaces in the ICU were: the infusion pump, bath cart, handwashing basin, monitor, and mechanical ventilator. Additional surfaces were collected in the AER, such as an examination light and a negatoscope. In the SC, additional surfaces collected were: surgical light, handwashing basin, sliding stretcher, surgical instrument table, and minibar door.

The samples were collected using a sterile swab moistened with sterile saline solution (0.85% NaCl), which was rubbed on each surface for five seconds. On flat surfaces, collection was performed in a delimited area of 100 cm^2, using plastic frames with this dimension.

After each collection, the frames underwent an aseptic cleaning procedure with 70% alcohol. On other surfaces that did not allow execution in this specific area, the swab was passed over the area randomly for the determined time.

After sample collection, the swabs were identified, placed inside a test tube containing 1 ml of sterile saline solution, and transported to the laboratory in Styrofoam boxes. The two hours before the start of sample processing was not exceeded.

Sample Processing and Bacterial Identification

In the laboratory, swabs with the samples were agitated for two minutes on a vortex mixer. Following this process, the contents of the test tubes (1 ml) were poured and streaked using the same collection swab across the entire surface of MacConkey agar plates and then incubated at 36 ± 1° C for 48 hours.

The grown colonies underwent a bacterial counting process for sample classification regarding bacterial load. The measure used to assess bacterial load was the Colony Forming Unit (CFU), which estimates the number of viable bacteria or yeasts capable of multiplying under controlled conditions on a Petri dish containing agar, i.e., each colony corresponds to one CFU.

The counting result was expressed in CFU/ml. Samples with a count less than or equal to 20 CFU/ml were classified as low bacterial load; greater than 20 CFU/ml and less than 50 CFU/ml, medium bacterial load; and greater than or equal to 50 CFU/ml were considered high bacterial load. Then, the colonies were isolated by streaking onto another plate with MacConkey agar.

At this stage, at least one representative colony of each morphotype was isolated. Subsequently, the colonies underwent Gram staining and TSI (Triple Sugar Iron) agar to differentiate enteric bacilli from non-fermentative bacilli based on the carbohydrate fermentation patterns and oxidase test.

Data analysis

The data were interpreted using descriptive statistical analysis with simple frequency distribution, expressed in percentages or simple or weighted arithmetic averages, with the aid of Microsoft Office Excel® software.

RESULTS

From the 150 samples collected and processed, 73 (49%) showed no microbiological growth of any kind. Accordingly, 77 (51%) samples exhibited some type of microbial growth. In 11 samples (7.3%), only filamentous fungi were visually detected; 58 samples (39%) showed only growth of CFUs, suggestive of bacteria or yeast; in 8 samples (5.3%), both filamentous fungi and CFUs were detected.

The 66 samples positive for CFU growth were visually evaluated for colony morphology. A total of 132 morphologically distinct colonies were included for Gram staining, among them, 55 (42%) were yeasts, while 77 (58%) were stained with the pink dye, indicating Gram-negative bacteria, and none were stained with purple dye.

Among the Gram-negative bacteria, 100% presented bacillary or coccobacillary morphology and the carbohydrate fermentation pattern showed that 51.6% of the isolates were fermentative bacilli, while 48.4% were considered Non-fermenting Gram-negative bacilli (NFGNB).

The growth proportion in each hospital ward analyzed was as follows: 60% of samples collected in the MC and AER showed CFUs growth; 53% of samples from PER and GSC; 47% of samples from PC and IPU; 35% of ICU collections; 33% of samples from OSC, and 15% of samples from SC had some type of growth.

Regarding the GNB growth only, among the 150 samples, 37 (25%) were positive. The ward with the highest quantity was AER, with 45% of positive samples.

The wards with the lowest GNB rates were ICU and SC, both with 10% GNB growth (Table 1).

Table 1. Assessment of the number of samples and contamination by hospital ward.

Hospital ward	Number of samples collected	Samples with CFU growth (%)		Samples with GNB growth (%)	
		Nº	%	Nº	%
Adult Emergency Room	20	12	60%	9	45%
Orthopedic Surgical Clinic	15	5	33%	5	33%
Medical Clinic	15	9	60%	4	27%
General Surgical Clinic	15	8	53%	4	27%
Pediatric Emergency Room	15	8	53%	4	27%
Pediatric Clinic	15	7	47%	4	27%
Infected Patients Unit	15	7	47%	3	20%
Intensive Care Unit	20	7	35%	2	10%
Surgical Center	20	3	15%	2	10%
Total	**150**	**66**	**45%**	**37**	**25%**

GNB: Gram-negative Bacilli; CFU: Colony Forming Unit.

In the evaluation of inanimate surfaces presented in Table 2, sink faucets and computer/phone keyboards were the most notable. These showed proportions greater than 75% of samples collected with GNB presence. However, most of the studied surfaces showed GNB growth proportions of less than 50% or no growth.

Another parameter evaluated was bacterial load. Most samples showed low bacterial loads. Samples from the surgical light, wall, door handle, light switch, medication box, and nursing station countertop exhibited only low bacterial loads.

Sink faucet and computer/phone keyboard surfaces showed medium and/or high loads in most samples. Within this parameter, beds can be highlighted in general, including those for short and long stays, and mattresses. Although all beds combined showed GNB growth in 36% of samples, the bacterial loads on these surfaces were considered medium or high in 62.5% of the samples.

Table 2. Assessment of the number of samples and contamination by inanimate surface.

Surface	Number of samples collected	Samples with GNB growth		Number of samples by bacterial loads		
		Nº	%	Low	Medium	High
Short-stay bed rails	2	2	100%	1	-	1
Sink faucets	9	8	89%	-	-	8
Telephone/computer keyboards	9	7	78%	3	3	1
Examination/Surgical light	2	1	50%	1	-	-
Long-stay bed rails	8	3	38%	2	-	1
Emergency carts	9	3	33%	2	1	-
Accompanying chairs	8	3	38%	2	-	1
IV poles	9	2	22%	1	-	1
Long-stay mattresses	8	2	25%	-	2	-
Short-stay mattresses	4	1	25%	-	-	1
Walls	9	1	11%	1	-	-
Door handles	9	1	11%	1	-	-
Light switches	9	1	11%	1	-	-
Medication boxes	9	1	11%	1	-	-
Nursing station countertop	9	1	11%	1	-	-
Mechanical ventilator	1	0	0%	-	-	-
Clipboards	9	0	0%	-	-	-
Minibar door	1	0	0%	-	-	-
Gas panels	9	0	0%	-	-	-
Negatoscope	1	0	0%	-	-	-
Monitor	1	0	0%	-	-	-
Surgical instrument table	1	0	0%	-	-	-
Sliding stretcher	1	0	0%	-	-	-
Handwashing basin	2	0	0%	-	-	-
Bath cart	1	0	0%	-	-	-
Infusion pump	1	0	0%	-	-	-
Medication preparation countertops	9	0	0%	-	-	-
Total	**150**	**37**	**20%**	**17**	**6**	**14**

GNB: Gram-negative Bacilli

DISCUSSION

This study revealed a contamination rate of 49% among the surfaces examined. Comparable results were observed in a Moroccan hospital, where 40% of 60 samples were contaminated, while Afle *et al.*[10] identified contamination in 65% of 160 surface samples.[9,10] Discrepancies in these percentages may be elucidated by variations in infection prevention and control practices, protocols, and management strategies adopted across different healthcare facilities and regions, all of which directly influence the burden of bacterial contamination.[11]

Our data revealed that 25% of the analyzed hospital inanimate surfaces were contaminated with GNB across all hospital wards. Despite Gram-positive cocci being the most prevalent microorganisms on hospital surfaces, GBN represents a significant portion of contaminants.[6,12]

Similar values were found in a study conducted in Iran, which observed GNB growth on 30.35% of the analyzed surfaces, and in a university hospital in Benin (West Africa) with a positivity rate of 35.8%.[10,13]

Conversely, a study carried out in a hospital in Ghana observed that 75% of surface contaminations were due to Gram-negative bacteria; however, this study focused solely on an emergency unit.[14]

Understanding which inanimate surfaces are contaminated and their proximity to hospitalized patients is of great epidemiological importance, as these bacteria often lead to nosocomial infections.[15]

Amare *et al.*[4] indicate that inanimate objects and medical devices are heavily contaminated with potentially pathogenic and multi-resistant bacteria. Through our presumptive assessment, half of the bacteria found belonged to the enterobacteria group, and the other half to the NFGNB group.

Within these two bacterial groups are the most critical priority pathogens for promoting research and development of new antibiotics, such as multi-resistant isolates of *A. baumannii*, *P. aeruginosa*, and Enterobacteriaceae.[16]

In a systematic review conducted by Dresch *et al.*,[12] these were the highlighted pathogens among the GNB that contaminate surfaces located in ICUs and operating rooms.

Regarding hospital wards, those with higher foot traffic were the most contaminated, including the Medical Clinic, Pediatric Clinic, and Adult and Pediatric Emergency wards.

This finding correlates with the high flow of people in these sectors, as there is a relationship between more contaminated surfaces and areas with high foot traffic.[17]

Conversely, it is observed that the surgical center and the ICU were the hospital wards with lower contamination rates, as they are sectors with more controlled foot traffic and rigorous infection prevention and control measures. Several studies also support lower contamination rates in surgical wards and ICUs.[9,11,18,19]

Surfaces with higher contamination rates (> 70%) included faucets, keyboards, and short-stay beds. This pattern is also shared with other studies.[5,19-21] Therefore, it can be concluded that contamination occurs primarily on objects with high manipulation frequency.[12]

Nazeri *et al.*[5] detected that more than two-thirds of all computer keyboards and electronic equipment in ICUs near patients' beds were contaminated with microorganisms that could contribute to developing HAIs. In another study, phones and patient beds showed the highest contamination levels, mainly with Enterobacteriaceae and *Pseudomonas* spp.[13] In the studies by Temesgen *et al.*[22] and Amare *et al.*,[4] the highest bacterial contamination was also identified in patients' beds.

Ledwoch *et al*.[23] detected Gram-negative bacteria in 45% of keyboard samples from three hospitals and a dental clinic in the United Kingdom, including strains that are multi-resistant to antibiotics. The simple use of sterile cloths moistened with sterile distilled water or sodium hypochlorite solution was ineffective in removing most contaminants, suggesting that these bacteria present on keyboards may be attached to the dry surface biofilms.

Firesbhat *et al*.[6] identified a predominance of *K. pneumoniae* and *A. baumannii* species in the sinks of a Specialized Hospital at the University of Gondar, Ethiopia.

Joachim *et al.*[19] indicated that sink/washing basins were frequently contaminated (74.2%) compared to other surfaces, but high contamination levels were also present on surfaces such as bed rails and keyboards.

Therefore, greater attention is needed to disinfect inanimate surfaces and restrict flow, as well as promote hand hygiene among healthcare staff, to break the chain of contamination from these etiological agents, and to assist in preventing nosocomial infections.[21]

In the study by van der Zwet *et al*.,[24] the same clones of *P. putida* and *Enterobacter cloacae* that were causing patient outbreaks were also present in sinks and taps in a hospital department in the Netherlands. Changing taps and siphons and intensifying the disinfection of sinks and drains was enough to contain the outbreak.

Limitations of this study include the fact that we used MacConkey agar to detect only Gram-negative bacterial contamination. This medium inhibits the growth of Gram-positive and some fastidious Gram-negative bacteria, such as those from *Pasteurellaceae* and *Neisseriaceae* families. If other growth media were used, contamination rates could be even higher. Furthermore, the study was limited to areas with patients, other sectors such as kitchen, laboratory, and sterilization service were not included. Additionally, despite not being this study's focus, it was impossible to completely identify the microorganisms due to the COVID-19 pandemic.

After collection in 2019, laboratory activities were suspended in 2020 and the processing and storage of samples was compromised.

CONCLUSION

In this study, it was observed that hospital wards with higher foot traffic are the most contaminated. Similarly, the Surgical center was the ward with the lowest growth due to restricted entry and notable emphasis on hand hygiene and material sterilization.

Consequently, contamination primarily occurs on objects with high manipulation frequency. Surfaces deserving of attention include sink faucets and computer/telephone keyboards, which are touched by the entire staff and serve as crucial reservoirs for pathogens.

Additionally, beds and mattresses should be highlighted as significant sources of microorganisms and in close contact with hospitalized patients. Hence, greater attention is needed to disinfect inanimate surfaces and restrict people's flow, alongside handwashing.

These practices should be reinforced in the most critical areas to break the chain of contamination from these etiological agents.

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Conflicts of Interest

The authors declare that there is no conflict of interest in carrying out this work.

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