Bacterial profile of surfaces and equipment of the Orthopedic Clinic of a University Hospital
Perfil bacteriano das superfícies e equipamentos da Clínica Ortopédica de um Hospital Universitário
Perfil bacteriano de las superficies y equipos de la Clínica Ortopédica de un Hospital Universitario

ABSTRACT

Background and Objectives: Healthcare-Associated Infections (HAIs) are an important public health problem that impacts negatively on hospital costs and patient prognosis. Given the importance of the hospital environment in the development of HAIs, the objective was to evaluate the bacterial profile on surfaces and equipment of the Orthopedic Clinic of the Hospital Universitário do Vale do São Francisco. Methods: This is a cross-sectional, descriptive, quantitative study. Samples were collected in 13 wards, each ward with four beds and one was chosen at random, where surfaces and equipment were sampled using swabs soaked in saline and a 1cm² filter paper mold to standardize the samples. After passing the swab, they were stored in a tube containing 5mL of BHI (Brain Heart Infusion) liquid medium. Then, samples were transported to the Clinical Analysis Laboratory/Microbiology Sector where the microbiological analyses were performed. Results: In total, 257 bacteria were observed, of which 5.11% were possible causes of hospital infection and 79% coagulase-negative Staphylococcus. Antibiograms of these were performed and different resistance profiles were found. The bathroom doorknob, a high-touch surface, presented the greatest variety of species among the evaluated surfaces. Conclusion: Surfaces and equipment of the evaluated clinic present possible bacteria that cause hospital infection with different profiles of antimicrobial resistance, contributing to possible cross infections.

Keywords: Bacterium. Bacterial infection. Hospital Infection. Equipment contamination. Patient safety.
Las infecciones asociadas a la asistencia sanitaria (IAAS) son un importante problema de salud pública que impacta negativamente en los costos hospitalarios y el pronóstico de los pacientes. Dada la importancia del entorno hospitalario en el desarrollo de las IAAS, el objetivo fue evaluar el perfil bacteriano en superficies y equipos de la Clínica Ortopédica del Hospital Universitario do Vale do São Francisco. **Métodos:** Se trata de un estudio transversal, descriptivo y cuantitativo. Las muestras se recolectaron en 13 salas, cada sala con cuatro camas y una se eligió al azar, donde se muestrearon las superficies y el equipo utilizando hisopos empapados en solución salina y un molde de papel de filtro de 1cm² para estandarizar las muestras. Después de pasar el hisopo, se almacenaron en un tubo que contenía 5ml de medio líquido BHI (infusión cerebro corazón). Luego, las muestras fueron transportadas al Laboratorio de Análisis Clínicos/Sector de Microbiología, donde se realizaron los análisis microbiológicos. **Resultados:** Se observó un total de 257 bacterias, de las cuales el 5,11% fueron posibles causas de infección hospitalaria y el 79% *Staphylococcus* coagulasa negativa. Se realizaron antibiogramas de estos y se encontraron diferentes perfiles de resistencia. La manija del baño, una superficie de alto tacto, presentó la mayor variedad de especies entre las superficies evaluadas. **Conclusiones:** Las superficies y el equipo de la clínica evaluada presentan posibles bacterias que causan infección hospitalaria con diferentes perfiles de resistencia a los antimicrobianos, lo que contribuye a posibles infecciones cruzadas.
when the technical standards of biosafety are disregarded and the health team do not understand the interference of the physical environment in the process of falling ill.\textsuperscript{7}

According to ANVISA, the deficient disinfection of surfaces and equipment and incorrect hand washing are the most common causes of HAI transmission.\textsuperscript{3} The hospital infrastructure houses reservoirs of pathogens, especially on surfaces and equipment. In this sense, health professionals represent potential means of transfer by cross-contamination and hands are the most common route.\textsuperscript{8} Orthopedic clinics are of great importance in this scenario due to the profile of patients who constantly perform invasive procedures, use immunosuppressants and need antibiotics as prophylactic treatments, thus constituting a greater risk for antimicrobial resistance. In association, specialized care regarding the handling of post-surgical dressings is needed, which depends on the use of professionals’ hands to be performed.\textsuperscript{9}

Among the main HAIs, those with a respiratory, urinary and hematological focus stand out, which evolve to increasingly aggravated clinical outcomes by the development of bacterial multidrug resistance.\textsuperscript{5} The process of bacterial resistance has grown in large proportions due to inadequate use of antibiotics and led to therapeutic limitations.\textsuperscript{10} Gram-positive microorganisms are the main involved in infections in the hospital setting, such as vancomycin-resistant Enterococcus (VRE) and methicillin-resistant Staphylococcus aureus (MRSA), gram-negative, such as the extended-spectrum beta-lactamase-producing enterobacteria (ESBL), bacteria of the CESP group – Citrobacter spp., Enterobacter spp., Serratia spp. and Providencia spp. - ESBL and AmpC producers, carbapenemase-producing Klebsiella pneumoniae, carbapenem-resistant Acinetobacter baumannii, Pseudomonas aeruginosa.\textsuperscript{11,12}

Given the importance of the hospital setting in the development of hospital infections, the objective was to evaluate the bacterial profile on surfaces and equipment at the Orthopedic Clinic of the Hospital Universitário do Vale do São Francisco in Petrolina (state of Pernambuco/PE) between March and April 2018.

METHODS

The experiment was conducted at the Orthopedic Clinic of the Hospital Universitário do Vale do São Francisco. This is a cross-sectional, descriptive, quantitative study developed with the aim to know the bacterial populations associated with surfaces and equipment. A letter of consent was requested to the teaching and research management of the Hospital Universitário do Vale do São Francisco for the development of the study. After analyzing the information about the research project, approval was issued. There was no need for submission to the Ethics Committee, as the study is limited to equipment and not aimed at interventions involving human beings.

At the Orthopedic Clinic, samples were collected in March and April 2018 in 13 wards, each ward having four beds and one was chosen at random. Samples were collected from the following surfaces and bed equipment: bed rail, bedside table, IV pole, wall, bed control, manual/digital crank, infusion pump, screens. Samples from the following surfaces and equipment were also collected in the wards: bathroom faucet and doorknob.

Surface and equipment samples were collected using swabs soaked in saline solution and a 1cm² filter paper mold was adopted for sample standardization purposes. After passing the swab on surfaces and equipment, they were stored in a tube containing 5mL of BHI (Brain Heart Infusion) liquid medium. Then, the tubes containing BHI and the swab were transported at room temperature to the Clinical Analysis Laboratory/Microbiology Sector, where microbiological analyzes were performed. Each surface and equipment were sampled at two random points.

In the laboratory, the BHI broths were incubated at 37°C for 48 hours. For bacterial isolation, samples were seeded in Blood Agar (BA) and incubated at 37°C for 24 hours. After the incubation period, gram staining and biochemical tests were performed to identify each species. For the identification of gram-positive cocci, a catalase test was performed. When catalase was positive, the coagulase test was performed and when catalase was negative, the kit for Enterococcus (PROBAC\textsuperscript{®}), bacitracin and optochin was used to identify the species. For the identification of gram-negative bacilli, kits for identification of enterobacteriaceae and/or glucose non-fermenter PROBAC\textsuperscript{®} were used, according to manufacturer’s instructions.

The identified bacteria were subjected to an antibiogram using the disc diffusion agar method according to CLSI instructions (2018) and the choice of antimicrobials was performed according to the isolated microorganism. The results were stored and analyzed in an electronic datasheet (Microsoft Excel\textsuperscript{®} 2003).

RESULTS

The total number of bacteria found, regardless of the ward, equipment and surfaces sampled was 257 (Figure 1). Thirteen (5.11%) are considered bacteria that can possibly cause nosocomial infections, namely: four isolates of Enterobacter cloacae, one of Serratia marcescens, one of Citrobacter youngae, four of Acinetobacter baumannii, two of Enterobacter aerogenes and one of

### Table 2. Isolated bacteria in the Orthopedic Clinic of the Hospital Universitário do Vale do São Francisco.

<table>
<thead>
<tr>
<th>Bacterioide isolado</th>
<th>Quantidade de bactérias isoladas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterioide isolado</td>
<td>Quantidade de bactérias isoladas</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>205</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>19</td>
</tr>
<tr>
<td>Citrobacter youngae</td>
<td>13</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>1</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>1</td>
</tr>
</tbody>
</table>

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Page 03 of 07

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Enterococcus faecium. The rest of isolated bacteria are in the group of coagulase negative Staphylococcus (205 isolates), so called because they do not form clots in rabbit plasma.14 Another 39 were isolated from other bacteria, possibly from the environment, so called in this study because they were not identified by the biochemical tests used in routine identification of bacteria of clinical interest and had the morphology of bacteria present in the environment, usually gram-positive bacilli.

In relation to the sampled equipment and surfaces, on the bed rail, bedside table, IV poles, the wall, bed control and on the hand crank of the digital and manual bed, coagulase negative Staphylococcus and other bacteria, probably from the environment, were found in all samples. In the infusion pump, in addition to these findings, Enterobacter cloacae was found, while in the faucet, in addition to the three different species of bacteria found in the infusion pump, Serratia marcescens was the differential finding. In the screens, in addition to bacteria from the environment and coagulase-negative Staphylococcus, Acinetobacter baumannii was found. The object with the greatest variety of bacterial species found was the bathroom doorknob, with bacteria from the environment, coagulase negative Staphylococcus, Acinetobacter baumannii, Enterobacter aerogenes, Citrobacter youngae, Enterococcus faecium (Table 1).

Regarding the resistance profile of bacteria that can possibly cause hospital infections, Table 2 displays that Enterobacter cloacae was 100% resistant to ampicillin, amoxicillin + clavulanate, piperacillin + tazobactam,
ciprofloxacin, 75% resistant to ciprofloxacin, 50% resistant to sulfamethoxazole + trimethoprim, ceftriaxone and cefepime and 100% sensitive to amikacin, ertapenem, meropenem, imipenem and levofloxacin. Enterobacter aerogenes isolates were 100% resistant to piperacillin + tazobactam, sulfamethoxazole + trimethoprim, gentamicin, ciprofloxacin, ceftriaxone, cefepime, cefazolin, ampicillin, amoxicillin, amoxicillin + clavulinate and 100% sensitive to amikacin, ertapenem, meropenem, imipenem and levofloxacin (Table 2).

The Serratia marcescens isolate showed resistance to amoxicillin, ampicillin + sulbactam, cefazolin and cefoxitin, intermediate resistance to imipenem and sensitivity to amikacin, cefepime, ceftriaxone, ertapenem, meropenem, gentamicin, levofloxacin, piperacillin + tazobactam, ticarcillin and sulfamethoxazole + trimethoprim (Table 2).

For Citrobacter youngae, resistance was observed only to amoxicillin and cefoxitin and sensitivity to amikacin, ampicillin + sulbactam, cefazolin, cefepime, ceftriaxone, ciprofloxacin, ertapenem, gentamicin, imipenem, levofloxacin, meropenem, piperacillin + tazobactam, ticarcillin and sulfamethoxazole + trimethoprim (Table 2).

Acinetobacter baumannii isolates were 75% resistant to piperacillin + tazobactam, 25% resistant to amoxicillin + tazobactam, and 100% sensitive to the rest of the antibiotics tested, namely: amikacin, cefepime, ceftazidime, ciprofloxacin, gentamicin, imipenem, levofloxacin, meropenem and sulfamethoxazole + trimethoprim (Table 2).

The Enterococcus faecium isolate was resistant to amoxicillin and penicillin G and sensitive to vancomycin, daptomycin and linezolid (Table 2).

The bacterial profile of the orthopedic clinic under study consists of high-risk bacteria and possible nosocomial infection-causing bacteria located on surfaces and equipment. Enterobacter cloacae and Acinetobacter baumannii species have higher prevalence and high resistance profile. Among the surfaces studied, the bathroom doorknob has the greatest variety of bacterial species. Thus, weaknesses are exposed both in the chance of healthcare-related contamination and in contaminating events related to the routine practiced by caregivers and/or visitors that visit the sector, contributing to possible cross-infection.

DISCUSSION

High-touch surfaces, such as those close to the patient, are considered to have a greater biological load, because of the great manipulation and possibility of contributing to secondary transmission through the hands of health professionals, caregivers and visitors. Research shows that inanimate objects of hospital environments are considered epicenters of bacterial contamination, therefore, it is necessary to effectively disinfect objects and equipment located close to the patient, leaving them microorganism-free.

As for surfaces and equipment that served to collect the samples (Table 1), the bed protection grid is constantly handled both by professionals, when performing procedures, and by caregivers, therefore, it is characterized as an important source of transmission. The bedside table used as support for personal objects of patients; the IV pole that serves to offer medications; the wall; the bed control to operate the bed movement; manual and digital bed crank; continuous infusion pump attached to a support for saline administration; faucet, bathroom doorknob and screens are also used collectively by patients in the same ward and part of the set of objects handled.

In a recent analysis performed to detect bacteria on inanimate surfaces in hospital environments, coagulase-negative Staphylococcus was found on inert surfaces such as the sink, wall, dressing cart and medication cart in the surgery sector. The observed result may be correlated with the fact that Staphylococcus are persistent colonizers of the hands. Thus, the hands of health professionals who manipulate patients and touch objects at the same time play an important role as a means of transmission to surfaces and equipment.

Among the infection-causing bacteria, Staphylococcus stands out. Although less virulent, they are associated with opportunistic infections that, in large part, colonize the skin. Depending on the patient’s immunological status and the treatment faced, these microorganisms can become pathogenic and increase the risk of hospital infection. They can cause infections associated with implanted devices and instruments, such as joint prostheses and intravascular probes, especially for young immunocompromised patients and older adults. The orthopedic clinical studied presents a profile with a majority of young patients affected by motorcycle accidents and older adult patients, generally victims of falls from their own height, both with a chance of implantation of prostheses.

The Enterobacteriaceae family group represents gram-negative bacilli with a natural habitat in the intestinal tract of humans and animals, therefore, they can also be called coliforms. These bacteria demonstrate considerable survival resistance outside their natural habitat. They withstand the most diverse environments for months, from dry surfaces to extreme situations and wet surfaces such as warm water pipes and hospital sinks. In a cohort study, the authors showed a high growth of carbapenem-resistant enterobacteria in ICU patients from hospitals surveyed between 2011 and 2017.

Species of the Enterobacter genus are lactose fermenters, many are encapsulated, producers of mucoid and mobile colonies, and may have a chromosomal beta-lactamase enzyme called AmpC, which makes them intrinsically resistant to amoxicillin and first and second generation cephalosporins. They undergo mutations and can overproduce beta-lactamase, conferring resistance to third-generation cephalosporins. In addition to these factors, the Enterobacter genus has a broad infectious spectrum in a nosocomial environment, making it a major public health problem.

Another bacterium of public health relevance present among isolates in the present study is Acinetobacter baumannii, an aerobic gram-negative, non-fermenting bacteria widely distributed in soil and water that can be isolated...
from samples of skin, blood, sputum, pleural fluid and urine, usually referenced in device-associated infections. The WHO highlights the bacterium as a critical priority pathogen given its proven resistance profile to carbapenems, leading to hospital outbreaks and culminating in sepsis, especially in critically ill patients. The production of enzymes called carbapenemases by these isolates reflects the main carbapenem resistance mechanism.

The therapeutic effect of carbapenems begins with the cell membrane of gram-negatives through transmembrane porins that act in the periplasmic environment by inhibiting the formation of the cell wall of bacteria, hence these are defined as bactercides. Although carbapenem antibiotics are the drugs with the greatest known antimicrobial spectrum and the bacteria isolated in this study have been 100% sensitive to carbapenems, the emergence of carbapenem resistance translates into a real public health problem.

In this context, *Serratia marcescens*, an enteric bacterium found to be opportunistic that presented intermediate carbapenem resistance and found in the faucet in this study, is commonly related to the prolonged use of venous/urethral catheters, causes infections related to the urinary tract, pneumonia and meningitis and can reach bloodstream evolving to sepsis. This is an important bacterium that emerged as a cause of hospital infections in recent years. A study was published indicating the transposition of a *bla* gene by a plasmid pKpQIL-IT, which involves transmission between *Klebsiella pneumoniae* and *Serratia marcescens* in the lower respiratory tract, presenting a new anatomical region for plasmid transmission events that eventually involves the gastrointestinal tract, in addition to acquisition of genomic determinants with carbapenem resistance, promoting potential therapeutic limitation.

*Citrobacter youngae*, still poorly studied, is part of gram-negative, citrate positive bacteria that do not de-carboxylate lysine, and may or may not ferment lactose. This species was found on the bathroom doorknob of the orthopedic clinic under study and is closely linked to urinary tract infections. It was certainly found on the bathroom doorknob due to lacking or inefficient hand washing.

In contrast, *Enterococcus faecium*, belonging to the group of gram-positive bacteria, are part of the enteric microbiota and transmitted from one patient to another primarily through the hands, and on certain occasions, from the hospital staff to patients through devices used in care, such as stethoscopes and thermometers or other high-touch surfaces, such as the bathroom doorknob. The presence of these microorganisms on this surface is closely related to users’ hand hygiene. In patients, the most common foci of infection are urinary tract, wounds, biliary tract and blood, which may develop endocarditis in adults.

The *Enterococcus* genus is noteworthy, as it is among the most frequent causes of hospital infections. Another important characteristic is the resistance of this genus to antimicrobials giving its competence in capturing resistance genes from other organisms, such as plasmids or even intrinsic resistance. The emergency prevention of resistant microorganisms in health institutions has been a major challenge. At the same time, continuing education in health, performing routine measures such as correct hand hygiene and standard disinfection of surfaces and equipment in accordance with regulatory standards are important in the services, and such postures have not always brought about the expected contribution to the control of HAIs.

Thus, knowledge of the prevalence and bacterial resistance in the hospital environment supports arguments for reflections and implementation of good conduct by the health team, in addition to allowing the construction of protocols aimed at clarifying caregivers and visitors about the good practices needed for appropriately monitoring the patients, thus avoiding harm to the health of those who are already vulnerable. From such attitudes, greater control in the process of dissemination of hospital infections and a progressive change in the evolution of intra-hospital bacterial resistance become possible.

REFERENCES


contribuído como co-orientadora e realizou a revisão e aprovação final do artigo; Sued Sheila Sarmento contribuiu como co-orientadora e realizou a revisão e aprovação final do artigo; Mirthes Maria Rodrigues Santana e Bruna Manuella Souza Silva contribuíram com a coleta dos dados, preparo dos materiais e identificação das bactérias; Carine Freitas e Silva contribuiu com a coleta dos dados, preparo dos materiais e identificação das bactérias e formatação do artigo.

Todos os autores aprovaram a versão final a ser publicada e são responsáveis por todos os aspectos do trabalho, incluindo a garantia de sua precisão e integridade.

**AUTHOR’S CONTRIBUTION**

Tatiana Carla Carvalho Amorim Guisande contribuiu com a organização dos dados e escrito do trabalho, pesquisadora principal na elaboração do trabalho; Sued Sheila Sarmento contribuiu como co-orientadora e realizou a revisão e aprovação final do artigo; Carine Rosa Naue contribuiu na identificação das bactérias e organização dos dados, e como orientadora além de realizar a revisão e aprovação final do artigo; Mirthes Maria Rodrigues Santana e Bruna Manuella Souza Silva contribuíram com a coleta dos dados, preparo dos materiais e identificação das bactérias; Carine Freitas e Silva contribuiu com a coleta dos dados, preparo dos materiais e identificação das bactérias e formatação do artigo.