

ORIGINAL ARTICLE

ISOLATED RESISTANT BACTERIA FROM INANIMATE SURFACES IN A PUBLIC HOSPITAL

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ABSTRACT

Objective: to describe the antimicrobial resistance profile of microorganisms present on inanimate surfaces.

Method: a descriptive study, conducted between February and June 2018. Forty microbiological samples were collected from surfaces of the Medical Clinic and Intensive Care Unit for Adults in a hospital located in Mato Grosso, Brazil. Microbial identification and sensitivity were performed by means of VITEK 2. The analysis of the resistance results was assessed according to the Clinical Laboratory Standards Institute guidelines.

Results: a total of 32 microorganisms were isolated from the 22 contaminated samples, the following among them: 14 (43.8%) Coagulase-Negative Staphylococcus, seven (21.9%) Acinetobacter baumanni complex, and three (9.4%) Enterobacter aerogenes. Of the Coagulase-Negative Staphylococcus, 11 (78.6%) presented multi-drug resistance to antimicrobial agents, and three (42.9%) of the Acinetobacter baumanni complex isolates were extremely resistant. Conclusion: this study evidenced the need for education with emphasis on proper and frequent

disinfection of surfaces and on hand hygiene after touching patients and surfaces close to them.

DESCRIPTORS: Hospital Infection; Patient Safety; Equipment Contamination; Microbial Resistance to Medications; Hospital Cleaning Service.

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RESUMEN:

Objetivo: describir el perfil de resistencia de microrganismos presentes en superficies inanimadas a agentes antimicrobianos. Método: estudio descriptivo realizado entre febrero y junio de 2018. Se recolectaron 40 muestras microbiológicas de superficies de la Clínica Médica y de la Unidad de Cuidados Intensivos para Adultos de un hospital de Mato Grosso, Brasil. Los procesos de identificación y sensibilidad microbiana se realizaron a través del dispositivo VITEK 2. El análisis de los resultados de resistencia se evaluó conforme a las directrices del Clinical Laboratory Standards Institute. Resultados: se aislaron 32 microrganismos de las 22 muestras contaminadas; entre ellos, hubo 14 (43,8%) Staphylococcus coagulasa negativa, siete (21,9%) Acinetobacter baumanni complex y tres (9,4%) Enterobacter aerogenes. Entre los Staphylococcus coagulasa negativa, 11 (78,6%) presentaron multi-resistencia a agentes antimicrobianos y tres (42,9%) de los aislados bacterianos de Acinetobacter baumanni complex fueron extremamente resistentes. Conclusión: se hizo evidente la necesidad de instrucción con énfasis en la correcta y frecuente desinfección de superficies y en el lavado de manos después de entrar en contacto con el paciente y con las superficies próximas al paciente.

DESCRIPTORES: Infección Hospitalaria; Seguridad del Paciente; Contaminación de Equipos; Resistencia Microbiana a Medicamentos; Servicio Hospitalario de Limpieza.

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INTRODUCTION

Healthcare Related Infections (HRIs) are among the most frequent adverse events in hospitalized patients, and result in high morbidity and mortality indexes worldwide, with a consequent increase in hospitalization times and hospital costs⁽¹⁾. A multicentric European study observed that, of the 151,709 patients hospitalized in Intensive Care Units (ICUs) for more than two days, 8.4% presented at least one HRI⁽²⁾. In Brazil, the relevance of the problem represented by these infections can be verified in a research study that observed 377 patients diagnosed with HRIs and found a mortality rate of 20.7%, from January 2015 to July 2016⁽³⁾.

Environmental contamination plays an important role in the acquisition of nosocomial pathogens, both by patients and by health professionals. These professionals often acquire pathogenic microorganisms from direct contact with patients, with body fluids, or with contaminated environmental surfaces⁽⁴⁾. Pathogenic agents can survive on environmental surfaces for days, weeks, and even months, when these surfaces are not properly cleaned and disinfected, thus substantially increasing the risk of HRIs⁽⁵⁾.

A number of studies confirm that multi-drug resistant bacteria have been reported as contaminating microorganisms of surfaces, telephones, keyboards, commonly used hospital equipment, and frequently touched surfaces in ICUs^(4,6-9). Health professionals generally underestimate the role of the environmental surfaces in the transmission of HRIs. In this sense, a number of studies show that the professionals commonly do not perform hand hygiene (HH) after contact with inanimate surfaces surrounding a patient, although it is a frequent indication at that moment in the care practice⁽¹⁰⁾.

It is emphasized that, especially in developing countries, there are few data on the extent of contamination and on the microbial profile of frequently used equipment and inanimate surfaces in health units⁽¹¹⁾. Therefore, the objective of this study was to describe the antimicrobial resistance profile of microorganisms present on inanimate environmental surfaces of hospital equipment in an Intensive Care Unit for adults and in the Medical Clinic of a hospital.

METHOD

This is a cross-sectional and descriptive study, conducted from February to June 2018, in a Medical Clinic unit and in an ICU for adults of a public hospital of Cuiabá-MT. These sectors were chosen because they are critical places for the dissemination of HRIs, since they are related to a large number of invasive procedures and to frequent use of antibiotics, in addition to housing patients with compromised immunity.

For microbiological assessment, 40 samples (20 from the Medical Clinic and 20 from the ICU) of environmental surfaces and hospital equipment were randomly selected. This study included inanimate surfaces and hospital equipment that belonged to the respective sectors and which presented high frequency of contact, either by professionals, patients, or companions. Samples were selected from keyboards, doorknobs, infusion pumps, multiparameter monitors, bedside tables, lateral bed railings, floors, taps, glucometers, medical record covers, trash can tops, diet vials, aspiration vials, soap dispensers, computer mice, and chair armrests. In addition, samples from the floor areas close to the patient's bed were included.

The collection procedures were conducted without informing the professionals about the environmental surface from which the sample would be collected, in order to reproduce the actual scenario of the practice. The data collection instrument was a questionnaire in which the researcher answered whether the surface or equipment belonged to the sector and whether it presented high frequency of contact with professionals, patients, or companions. In addition to that, this instrument identified the date, collection sector, and inanimate surface or equipment from which the sample was collected.

Sample collection was performed using sterilized swabs with Stuart transport medium, which were rubbed against the surfaces of the objects and their saliences, duly coded, and sent for processing and analysis in a microbiology laboratory. The swabs were then cultivated by direct inoculation in Petri's plates containing selective media for the growth of microorganisms: selective agar for *Enterococcus*, Cetrimide agar, eosin Methylene Blue agar, Mueller-Hinton agar supplemented with 4% NaCl and 6 μ g/mL of oxacillin, Mueller-Hinton agar supplemented with 70 μ g/mL of zinc sulphate and 1 μ g/mL of meropenem, and potato dextrose agar with 40 mg/L gentamicin. The plates were incubated at 36°C, and readings occurred after 24, 48 and 72 hours. The colonies were first identified by their morphotinctorial characteristics and by classical microbiological techniques.

The identification and the antimicrobial susceptibility test of isolates were automatically performed using the VITEK 2® equipment (bioMerieux®, Marcy L'Etoile, France) according to the manufacturer's instructions. The Minimum Inhibitory Concentration (MIC) for analysis of the resistance results was assessed according to the 2018 Guidelines of the Clinical Laboratory Standards Institute⁽¹²⁾.

The data collected were double-typed in an Excel spreadsheet to avoid transcription errors. The R software⁽¹³⁾ was used for descriptive and inferential analysis. The Z test was used to conduct a comparative analysis of the proportions of microorganism growth between the sectors analyzed. The significance level considered in the analysis was 5%.

The study was submitted to the Research Ethics Committee of the Julio Muller University Hospital and approved under Opinion number 2,441,333.

RESULTS

Of the 40 collected samples, 22 (55%) presented positive growth for at least one microorganism. The description of the sampled environmental surfaces and equipment, as well as the number of positive samples for bacterial growth and identification and number of isolated bacteria, are presented in Table 1.

Table 1 - Type and number of inanimate surfaces sampled (n=40) for microorganisms. Cuiabá, MT, Brazil, 2018 (continues)

Inanimate surfaces	Positive samples for bacterial growth	Isolated microorganisms
Floor (n=5)	4	CoNS (2), Acinetobacter baumanni complex (3), Enterobacter aerogenes (1), Enterococcus hirae (1)
Bedside table (n=1)	1	CoNS (1), Acinetobacter baumanni complex (1), Enterococcus faecium (1)
Defective trash can top (n=2)	1	CoNS (1), Acinetobacter baumanni (1), Enterobacter aerogenes (1)

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Multiparameter monitor (n=2)	1	Acinetobacter baumanni complex (1)
Soap dispenser (n=1)	1	Acinetobacter baumanni complex (1)
Bed railing (n=4)	2	CoNS (2), Enterobacter asburiae (1), Enterobacter aerogenes (1)
Tap in the medication preparation room (n=2)	2	CoNS (1) e Sphingomonas paucimobilis (1), Roseomonas gilardii (1)
Doorknob (n=4)	2	CoNS (2), Burkholderia spp (1)
Keyboard (n=3)	2	CoNS (1), Sphingomonas paucimobilis (1)
Telephone (n=2)	1	Staphylococcus aureus (1)
Chair armrest (n=1)	1	Staphylococcus aureus (1)
Infusion pump (n=5)	3	CoNS (3)
Portable glucometer (n=1)	1	CoNS (1)
Medication preparation bench (n=2)	-	_
Computer mouse (n=2)	-	_
Medical record cover (n=1)	-	
Aspiration vial (n=1)	-	
Enteral diet vial (n=1)	-	_

CoNS: Coagulase-Negative Staphylococcus

Note: More than one microorganism was present in some samples. Source: The authors (2018).

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From these samples, 32 bacterial species were isolated, consisting in 14 (43.8%) positive samples for Coagulase-Negative *Staphylococcus* (CoNS), seven (21.9%) for *Acinetobacter baumanni complex*, three (9.4%) for *Enterobacter aerogenes*, and two (6.3%) for *Staphylococcus aureus*. In addition to that, one isolate of each of the following bacteria was identified on sampled inanimate environmental surfaces and hospital equipment: *Enterococcus hirae*, *Enterococcus faecium*, *Enterobacter asburie*, *Sphingomonas paucimobilis*, *Roseomonas gilardii* and *Burkholderia spp*. It is important to highlight that the VITEK 2® automated identification method identifies only Acinetobacter baumanni complex; thus, any of these species can be pathogenic: A. baumannii, A. calcoaceticus, A. nosocomialis, *A. dijkshoorniae*, and *A. pittii*⁽¹⁴⁾.

Among the samples collected with CoNS isolates, five (35.7%) presented Staphylococcus haemolyticus, three (21.4%) Staphylococcus homnis spp., two (14.3%) Staphylococcus epidermidis, and Staphylococcus saprophyticus was observed in two samples (14.3%). In addition, Staphylococcus lentus and Staphylococcus captis isolates were found in one (7.1%) sample each.

In relation to antimicrobial susceptibility, all CoNS isolates presented sensitivity to vancomycin, linezolid, daptomycin, teicoplanin, tigecycline, nitrofurantoin, and streptomycin. However, it is worth noting that, according to Table 2, all CoNS isolates were resistant to benzylpenicillin and, of the total of 14 isolates, only three were susceptible to oxacillin and clindamycin, corresponding to 78.6% resistance of the CoNS to each of these antibiotics. Of the two *S. aureus* isolates obtained from the collected samples, one was resistant to benzylpenicillin, oxacillin, erythromycin, gentamicin, levofloxacin, and clindamycin, whereas the other *S. aureus* isolate was only resistant to benzylpenicillin (Table 2).

Table 2 - Resistance profile of Gram-positive bacteria (n=18) found in samples of inanimate surfaces. Cuiabá, MT, Brazil, 2018

Variables	CoNS (n=14)			lococcus is (n=2)		ococcus m (n=1)	Enterococcus hirae (n=1)	
	f	%	f	%	f	%	f	%
Benzylpenicillin	14	100	2	100	1	100	0	0
Oxacillin	11	78,6	1	50	0	0	0	0
Gentamicin	6	42,9	1	50	0	0	0	0
Levofloxacin	10	71,4	1	50	1	100	0	0
Erythromycin	9	64,3	1	50	1	100	0	0
Clindamycin	11	78,6	1	50	1	100	1	100
Rifampicin	4	28,6	0	50	0	0	0	0
Trimethroprim/ Sulfamethoxazole	6	42,9	1	50	1	100	0	0
Ampicillin	-	-	-	-	1	100	0	0
Streptomycin	-	-	-	-	1	100	0	0

- Sensitivity test not performed.

Source: The authors (2018).

Table 3 shows the resistance profile of the Gram-negative bacilli obtained from the researched samples. It is emphasized that, of the seven *Acinetobacter baumanni complex* isolates, three presented resistance to carbapenems (meropenem and imipenem), but were sensitive to colistin. The susceptibility test was not performed for the *Sphingomonas paucimobilis*, *Roseomonas gilardii* and *Burkholderia* isolates.

Table 3 - Resistance profile of Gram-negative bacteria (n=11) found in samples of inanimate surfaces. Cuiabá, MT, Brazil, 2018 (continues)

Variables	baumani	tobacter ni complex =7)	aero	obacter genes =3)	Enterobacter asburiae (n=1)		
	f	%	f	%	f	%	
Ampicillin	7	100	3	100	0	0	
Cefuroxime	7	100	3	100	0	0	
Cefuroxime axetil	7	100	3	100	0	0	
Cefoxitin	7	100	3	100	1	100	
Ceftazidime	3	42,9	0	0	0	0	
Ceftriaxone	3	42,9	0	0	0	0	
Ampicillin/Sulbactam	2	28,6	3	100	0	0	
Piperacillin/Tazobactam	4	57,1	0	0	0	0	
Cefepime	3	42,9	0	0	0	0	

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Imipenem	3	42,9	0	0	0	0
Meropenem	3	42,9	0	0	0	0
Amikacin	3	42,9	0	0	0	0
Gentamicin	1	14,3	0	0	0	0
Ciprofloxacin	3	42,9	0	0	0	0
Tigecycline	1	14,3	0	0	0	0
Colistin	0	0	0	0	0	0

Source: The authors (2018).

Chart 1 presents the profile of the bacteria isolated from the study samples that were multi-drug resistant (MDR) and extensively drug-resistant (XDR) to antimicrobial agents. MDR is defined as the resistance acquired to at least one agent in three or more antimicrobial categories. XDR, in turn, is conceptualized as the resistance to at least one agent in all antimicrobial categories, except for only one or two categories⁽¹⁵⁾.

Chart 1 – Antimicrobial resistance profile of MDR or XDR microorganisms. Cuiabá, MT, Brazil, 2018

Bacterial isolate	Number of MDR or XDR microorganisms	Resistance pattern
Acinetobacter baumanni complex	XDR - 3/7 (42,9%)	AMP, APS (beta-lactam), CEF, CFT (second generation cephalosporins), CFZ, CFX (third generation cephalosporins), PIT (beta-lactam β-lactamase inhibitors) CPM-cefepime (fourth generation cephalosporin), IP, MPM (carbapenems), AMI, GEN (aminoglycosides), CIP (quinolone)
Staphylococcus coagulase negativa	MDR - 10/14 (71,4%)	BEN (beta-lactam), OXA (penicillinase-resistant beta-lactam), CLI (lincosamide), LVX (fluoroquinolone), ERY (macrolide)
Staphylococcus aureus	MDR - 1/2 (50%)	BEN (beta-lactam), OXA (penicillinase-resistant beta-lactam), CLI (lincosamide), LVX (fluoroquinolone), ERY (macrolide), GEN (aminoglycoside)
Enterococcus faecium	MDR - 1/1 (100%)	ERY (macrolide), LVX (fluoroquinolone), CLI (lincosamide), TMP/ SMX (sulfonamide + diaminopyrimidine), STP (aminoglycoside)

MDR: Multi-Drug Resistant, XDR: Extensively Drug-Resistant, BEN: Benzylpenicillin, OXA: Oxacillin, CLI: Clindamycin, LVX: Levofloxacin, ERY: Erythromicin, AMP: Ampicillin, APS: Ampicillin/Sulbactam, CEF: Cefuroxime, CFT: Cefoxitin, CFZ: Ceftazidime, CFX: Ceftriaxone, CPM: Cefepime, PIT: Piperacillin/Tazobactam, GEN: Gentamicin, CIP: Ciprofloxacin, TMP/SMX: Trimethoprim/ Sulfamethoxazole, STP: Streptromycin, AMI: Amicacin, MPM: Meropenem, IP: Imipenem. Source: The authors (2018).

It is worth noting that 10 (71.4%) CoNS isolates presented an MDR phenotypic profile and that three samples presented XDR *A. baumanni complex* isolates, showing susceptibility only to tigecycline and colistin. The three XDR *A. baumanni complex* isolates were obtained from environments close to the patient in samples of the bedside table, floor, and multiparameter monitor. The MDR *E. faecium* isolate was found in a sample from

the patient's bedside table. Furthermore, the sample collected from a telephone at the Nursing station of the Medical Clinic was positive for MDR *S. aureus*. The *E. aerogenes*, *E. asburie* and *Enterococcus hirae* isolates did not present an MDR phenotypic profile.

According to Table 4, the Medical Clinic was the sector with the largest number of contaminated samples, with 65% showing growth of microorganisms; however, the difference between sectors was not statistically significant (p=0.34). It is noted that six positive samples for *Acinetobacter baumanii complex* were found in the Medical Clinic, of which three were XDR isolates.

Table 4 - Distribution of the microorganisms present in the environment according to the hospital sectors. Cuiabá, MT, Brazil, 2018

Variables		5N 14)		•NS •14)		RSA =1)		5 SA =1)	fae	E. cium =1)		nirae =1)	gro	ith wth 22)	gro	hout wth =18)
	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%
Medical Clinic (n=20)	10	50	9	45	1	5	-	-	1	5	1	5	13	65	7	35
ICU for Adults (n=20)	4	20	5	25	-	-	1	5	-	-	-	-	9	45	11	55

Source: The authors (2018).

ICU: Intensive Care Unit; GNB: Gram-Negative Bacillus; CoNS: Coagulase Negative Staphylococcus; MRSA: Methicillin/oxacillin-Resistant Staphylococcus aureus; MSSA: Methicillin/oxacillin-Sensitive Staphylococcus aureus.

Note: More than one microorganism was present in some samples.

DISCUSSION

The sample with the greatest variety of microorganisms was found on a bedside table, including E. faecium, MDR CoNS, and XDR A. *baumanni* bacterial isolates. This high contamination in the bedside table was found in previous research studies^(4,13). A study conducted in Ethiopia found 13 microorganism isolates in 27 samples from bedside tables, as follows: one CoNS, three *Escherichia coli*, two *Klebsiella*, two *Proteus*, two *Pseudomonas aeruginosa*, two *Serratia*, and one *S. aureus*⁽¹¹⁾. In Iran, a study with a larger sample of bedside tables (124) verified that there were 22.58% cases of *S. epidermidis*, 10.48% of *A. baumanni*, and 5.65% of *S. aureus*⁽⁴⁾.

Bedside railings, multiparameter monitors and infusion pumps also presented microorganisms frequently related to HRIs and relevant to increased morbidity and mortality rates, such as carbapenem-resistant *A. baumanni*, *Enterobacter spp*, and oxacillin-resistant CoNS. In similar studies, pathogenic agents were also verified in the patient's environment^(4,6,11,16). A previous study found 14 (10.9%) cases of *S. aureus*, 25 (19.5%) of CoNS, and 16 (12.5%) of *Acinetobacter spp* in 128 bed samples⁽⁴⁾.

In this context, there is a concerning number of pathogenic bacteria on environmental surfaces and hospital equipment around the patients, since they are frequently touched and mutually contacted by professionals, patients and visitors, thus favoring cross-transmission⁽¹⁷⁾. An observational research study verified that the two items most mutually

touched by professionals, patients, and visitors were bedside railings, with a mean of 13.6 contact-episodes per hour, followed by bedside tables, with 12.3 contact-episodes per hour⁽¹⁷⁾.

The devices that support the work process of the health and management teams, such as telephones, chair armrests, computer keyboards, and soap dispenser in the medication preparation room, presented positive samples for potentially pathogenic bacteria, including MDR *S. aureus*. This fact indicates that the professionals can be vectors of potential pathogens from patients to more distant care environments.

The contamination of inanimate surfaces located outside the patient's environment corroborates with the results of research studies conducted in Brazilian and Iranian hospitals^(4,18). Even when these surfaces and equipment are not being used in direct patient care, they are able to colonize and infect patients through the professionals' hands and have high potential for microbial contamination spread because they consist of collectively used equipment⁽¹⁸⁾.

With regard to the samples collected from trash can tops with defective lifting mechanisms, *A. baumanni complex* and *Staphylococcus saprophyticus* isolates were observed. Since these tops needed to be manually lifted after HH, the professionals could consequently be contaminated with these bacteria and spread them throughout the hospital. It is worth noting that the initiatives that managed to reduce the rates of HRIs mainly invested in infrastructure⁽¹⁹⁾.

The samples from the floor presented a diversity of bacteria, corroborating previous research studies^(11,20). Despite the floor having presented high contamination levels, the cleaning teams usually show to attribute little relevance to floor disinfection. However, floors are a potential transmission reservoir, since they frequently come into contact with objects that the patients or professionals will later touch with their hands⁽²⁰⁾.

In this sense, a research study conducted in five hospitals with 318 samples of patient room floors identified contamination with *Clostridium difficile*, methicillin/oxacillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant *Enterococcus* (VRE). The same study verified that there was transfer of pathogens from the floor to objects that came into contact with it, and from these objects to hands and gloves, with MRSA being found in 18% of the samples, VRE in 6%, and *C. difficile* in 3%⁽²⁰⁾.

With regard to the CoNS isolates found in the samples, a high resistance rate (71.4%) to oxacillin was verified. The large number of samples with CoNS isolates with an MDR phenotype stands out, since this characteristic confers pathogenicity to these bacteria, which becomes a challenge to the treatment of hospital-acquired infections⁽²¹⁾.

Contamination with MDR CoNS isolates in the hospital environment is also concerning, because these microorganisms can be important gene reservoirs of antimicrobial resistance, which can be transferred across staphylococcus species⁽²²⁾. It is worth noting that multidrug resistance was observed in an oxacillin-resistant *S. aureus* isolate, which in turn causes bacteremias that are related to higher mortality rates and prolonged hospital stays⁽²³⁾.

As for GNB susceptibility, it is important to point out the severity of the three samples that presented XDR *A. baumanni complex* isolates, since the infections by these microorganisms cause significant morbidity and mortality among hospitalized patients and lack an established optimal treatment⁽²³⁾. The high resistance found in these isolates can be related to the generalized use of carbapenems⁽²⁴⁾.

Regarding the presence of samples with bacterial isolates in the different hospital sectors, a previous research study verified a higher rate of contamination with oxacillin-resistant CoNS in the Medical Clinic when compared to the ICU, which corroborates the findings of this study⁽²⁵⁾. In this sense, it could be inferred that the Medical Clinic commonly has a larger number of patients and relatives and understaffing of nursing and cleaning

professionals, which can explain the results obtained. A recent Brazilian study verified the following as potential factors for unsatisfactory environmental cleaning: low efficiency of the biocide used, contaminated wipes, variable compliance with the HH procedure, and stability of several bacteria genera to disinfection⁽²⁶⁾.

Regarding the limitation of this research, the reduced number of samples and collection sectors must me mentioned, which precludes generalization of the results.

CONCLUSION

It is expected that this research may be used by the teams involved in the Prevention and Control of Healthcare-Related Infections, for the planning of educational programs that emphasize the importance of proper, frequent and routine cleaning and disinfection of equipment and inanimate surfaces of the health units. In addition, the presence of resistant bacteria on surfaces from environments more distant to the patients reinforces the importance of hand hygiene after touching patients and after contact with inanimate surfaces surrounding them, an attitude that must also be strongly encouraged by the continuing education teams.

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