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Article

Microbiological Analysis of Surgeons' Hands in a Public Hospital in São Luis, Maranhão State, Brazil

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Abstract: Antisepsis of the hands of medical personnel is one of the most important steps in the process of patient care, since direct contact can cause the cross-transfer of potentially pathogenic microorganisms at surgical sites.This study aimed to analyze the prevalence of microorganisms on the hands of 131 surgeons in a university hospital before the surgical procedure. Swabs were collected from each clinician's hands before and after handwashing. The samples were placed in a transport medium and immediately delivered to a private Clinical Analysis Laboratory from São Luis-Maranhão. The microorganisms were identified by ionization source mass spectrometry and matrix-assisted laser desorption (MALDI-TOF), and antibiotic susceptibility tests (AST) were performed using the Vitek2 automated system. The results showed a high frequency (100%) of microorganisms before handwashing, but after surgical antisepsis, the rate dropped significantly ($p<0.05$) to 27.5%. Gram-positive cocci and rods were the most common microbes, followed by gram-negative bacilli species. The effectiveness of hand antisepsis was 72.5%. The ideal would be 100% efficacy, that is, 0% microorganisms in all surgeons. It is logical that the presence of any organism, no matter how infrequent it may be, will always represent a great risk of postoperative infection for any patient.

Keywords: hands; antisepsis; surgeons; surgical sites; microbiology

1. Introduction

Health care-associated infections (HAIs) (also referred to as "nosocomial" or "hospital" infections) are a serious public health problem that has increased considerably in recent years [1,2]. Studies have shown that these infections affect millions of patients worldwide every year [3–5].

The occurrence of HAIs is considered one of the most prevalent adverse events in hospital care that has caused negative impacts on the health of patients hospitalized in various sectors, especially in those who occupy intensive care unit (ICU) beds [6,7].

Some important factors may be associated with the high prevalence of HAIs in the hospital environment, including a long period of hospitalization; major surgical procedures; use of invasive instruments; indiscriminate use of antibiotics; conditions inherent to the patient, such as the existence of chronic diseases (diabetes, skin wounds); characteristics related to patient care sectors, such as intensive care units (ICUs); and use of inadequate hand hygiene techniques by visitors, nursing professionals, intensive care physicians, and surgeons [8,9].

In addition to the above factors, patient infection can also occur through some invasive procedures, such as intravenous drug application, insertion of catheters and drains, percutaneous

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tracheostomy, prosthetic devices, endotracheal intubation, mechanical ventilation, oral routes, and surgery [10–12].

It is noteworthy that the hospital environment offers specific conditions for circulation and prolonged persistence for many species of pathogenic microorganisms, including gram-negative (GN) bacteria with multidrug resistance (MDR) to drugs used in clinical practice [13,14].

Studies have shown that the main species detected in HAIs are gram-positive microorganisms, especially members of the *Staphylococcus* genus, such as methicillin-resistant *Staphylococcus aureus* (MRSA), and other gram-negative species, including *Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae,* and some members of the carbapenem-resistant/carbapenemase-producing *Enterobacteriaceae* family (e.g., *Escherichia coli, Enterobacter* spp., *Serratia* spp.). These pathogens are involved in an increased mortality rate, length of hospital stay, and financial burden for patients, families, and health systems [2,9,12,15–17].

In the hospital environment, microorganisms that cause HAIs can be transmitted mainly by the contaminated hands of health professionals, which act as important vehicles for the transport of pathogenic microorganisms to susceptible patients [4,6–8].

Each individual has a transient microbiota that changes over time, depending on environmental conditions, availability of nutrition and/or stage of growth and health, or even the diurnal rhythms of the hosts [18,19]. In addition to this transient microbiota, health workers can be contaminated during the handling of patients colonized by pathogens or by microorganisms that can remain viable on equipment, on the surfaces of bedroom and bathroom furniture, on instrument carts, bed rails, bed surfaces, bedside tables, intravenous pumps, and handwashing faucets [12,13,20,21]. In this sense, outbreaks and even continuous sporadic transmission of pathogenic microorganisms with MDR profiles can occur through the hands of health care personnel to inpatients, especially those in ICUs [20].

Handwashing is a prerequisite for reducing the occurrence of HAI [18]. Although no protocol guarantees the elimination of 100% of the microorganisms present in the hands of health professionals, the simple act of washing hands by visitors, nurses, and other members of health care teams may reduce the risk of transmission of HAI [22]. During this process, transient flora that colonize the surface of the skin can be removed, and the resident microbiota of the skin of the hands and forearms of the professionals who participate in the surgeries can be reduced [23].

The antisepsis performed by the ordinary citizen and/or visitor to the hospitalized patient is simpler than that used by health professionals since the latter must do so judiciously, before and after direct or indirect contact with patients, following the protocol adopted by the hospital [24]. In Brazil, surgeons have used the guidelines for safe surgery of the World Health Organization [25]. Despite the wide use of these guidelines, the complete elimination of microorganisms from the hands of health professionals remains a major challenge, implying the need for changes in habits and educational measures.

In this study, we verified that the hands of medical staff are important vehicles for the transmission of pathogenic microorganisms into the hospital environment, which can cause infections in hospital patients in various care settings. Our findings suggest that there is an urgent need to improve the integration of the six international patient safety targets among surgeons in the hospital under investigation.

2. Materials and Methods

2.1. Experimental design and sample collection

This was an observational analytical epidemiological study of the cross-sectional type with an applied nature and a quantitative and qualitative approach. A total of 150 doctors were selected by lot among surgical staff and residents who work in the adult surgical center of the Presidente Dutra Unit of the University Hospital of the Federal University of Maranhão. Of this total, 19 were excluded for presenting incomplete data, leaving 131 surgeons eligible for the survey. Initially, these selected

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surgeons were informed about the research, signed the informed consent form and then answered a structured epidemiological questionnaire.

Collections were carried out between May and September 2022, always by a single researcher. On another random day, both hands of these chosen surgeons were collected before brushing; swabbing with a sterile cotton swab soaked in sterile saline solution was performed on the pulps of the fingers, interdigital region, palms, and the wrists and back of the hands, and the sample was then placed in Stuart transport medium. Afterward, their brushing was observed in terms of sequence, time, and product used, and a swab was collected from both wet hands after antisepsis in the same direction as the brushing and placed in Stuart's medium, always before the first surgery of the shift. All samples were identified and stored in a thermal box until they were taken the same day for microbiology processing in a private laboratory in the city. Each week, a sample from the toilets was also sent for analysis.

2.2. Microbiological identification and antimicrobial susceptibility tests

The isolation, identification, and susceptibility tests of the microorganisms present in the samples collected from the hands of the physicians were carried out in a laboratory belonging to the private network of clinical analysis in São Luis-MA, according to the standard norms of microbiology.

The identification of microorganisms was performed by ionization source mass spectrometry and matrix-assisted laser desorption (MALDI-TOF), and antibiotic susceptibility tests (AST N408) were performed using the Vitek2 automated system (bioMérieux, Inc., Durham, NC) and BD Phoenix (AST-Pmic89). The cutoff criteria used for the interpretation of the susceptibility tests for determination of the minimum inhibitory concentration (MIC) and diameters of halos were based on the Brazilian Committee for Antimicrobial Sensitivity Tests and European Committee for Antimicrobial Test (BrCAst-EUCAST) [26].

2.3. Statistical analysis

Data analysis was performed using IBM SPSS Statistics 22 software [27]. Initially, descriptive statistics of the analyzed variables were performed using frequency tables and graphs. For numerical variables, the minimum, maximum, mean, and standard deviation estimates were calculated. Subsequently, the normality of numerical variables (age, time since formation, number of species before and after antisepsis) was evaluated using the Shapiro Wilk test, as all of them presented a p value <0.05, indicating that they did not have a normal distribution and therefore should be evaluated through nonparametric tests.

The evaluation of the presence or absence of microorganisms before and after hand antisepsis was performed using the McNemar nonparametric test. For the evaluation of the number of species per individual at both times (before and after) concerning hand antisepsis, the Wilcoxon nonparametric test was used. The evaluation of the effect of the surgeon's gender with numerical variables was performed using the nonparametric Mann–Whitney test. The association of nominal variables with the 2 groups (before and after antisepsis) was performed using the chi-square test for independence. In all tests, the significance level (α) used was 5%, that is, differences were considered significant when p was <0.05.

2.4. Ethical aspects of research

This project was submitted and approved by the Research Ethics Committee of the University Hospital of the Federal University of Maranhão under opinion number 2.638.389/2018. All participants read, approved and signed the informed consent form.

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3. Results

3.1. Microbiological identification of microorganism species

In this study, we evaluated the presence of microorganisms in the hands of 131 surgeons working in different specialties at a university hospital in the city of São Luís-MA. The results of the microbiological analyses of the samples collected before handwashing showed that there was growth of microorganisms in all 131 samples, with a frequency of 283 isolates belonging to 68 different species of microorganisms, among which 217 (76.7%) were gram-positive bacteria, 55 (19.4%) were gram-negative bacteria, and 11 (3.9%) were fungi (Table 1).

In both cases, there was a predominance of gram-positive microorganisms on the evaluated hands; the presence of 133 isolates distributed across 11 different species of the *Staphylococci* genus corresponded to 61.3% of the isolates, followed by 44 strains of *Micrococcus luteus*, which represented 20.26% of the isolates recovered before washing hands.

After hand antisepsis, 25 isolates of *Staphylococcus* spp. were detected, representing 92.5% of all microorganisms obtained after the washing procedure, and the most-detected isolates were *Staphylococcus warneri* and *Staphylococcus capitis*. *Micrococcus luteus* was also prevalent after antisepsis (Table 1).

Table 1. Frequency distribution of microorganism species isolated before surgical antisepsis of the hands.

gram-positive	n.	%	gram-negative	n.	%	fungus	n.	%
Aerococcus viridans	1	0.45	*Acinetobacter baumanii*	5	9.11	*Aspegillus versicolor*	2	18.20
Bacillus simplex	1	0.45	*Acinetobacter ursingii*	2	3.63	*Candida haemulonii*	1	9.10
Brevibacterium casei	2	0.90	*Acinetobacter variabilis*	2	3.63	*Candida parapsilosis*	4	36.30
Brevibacterium celere	7	3.21	*Aspergillus fumigatus*	1	1.82	*Pichia kudriavzevii (C. krusei)*	1	9.10
B. ravenspurgense	1	0.45	*Citrobacter sedlakii*	1	1.82	*Rhodothorula mucilaginosa*	1	9.10
Corynebacterium casei	1	0.45	*Enterobacter cloacae*	2	3.63	*Trichosporon asahii*	1	9.10
C. minutissimum	1	0.45	*Enterococcus faecalis*	1	1.82	*Trichosporum japonicum*	1	9.10
C. simulans	1	0.45	*Klebsiella pneumoniae*	1	1.82			
Dermacoccus nishinomiyaensis	2	0.90	*Methylobacterium radiotolerans*	1	1.82			
Kocuria kristinae	1	0.45	*Moraxela osloensis*	2	3.63			
Kocuria marina	2	0.90	*Moraxella spp*	1	1.82			
Micrococcus luteus	44	20.26	*Pantoea dispersa*	2	3.63			
Oceanobacillus onocorhynchi	1	0.45	*Pantoea septica*	2	3.63			
Penicillium spp	1	0.45	*Pseudomonas aeruginosa*	1	1.82			
Rhodococcus equi	1	0.45	*Pseudomonas extremorientalis*	1	1.82			
Bacillus cereus	7	3.21	*Pseudomonas gessardi*	1	1.82			
Bacillus clausii	1	0.45	*Pseudomonas stutzeri*	2	3.63			
Bacillus megaterium	1	0.45	*Serratia marcescens*	2	3.63			
Bacillus pumilus	1	0.45	*Burkholderia lata*	1	1.82			
B. amyloliquefaciens	1	0.45	*Mixta calida (Pantoea callida)*	2	3.63			
B. thermoamylovorans	1	0.45	*Neisseria subflava*	1	1.82			
Lactobacillus paracasei	1	0.45	*Rhizobium radiobacter*	1	1.82			
Staphylococcus aureus	6	2.76	*Roseomonas mucosa*	5	9.11			

S. arlettae	1	0.45	*Stenotrophomonas maltophilia*	15	27.27			
S. capitis	12	5.52						
S.caprae	1	0.45						
S. cohnii	10	4.60						
S. epidemidis	40	18.40						
S. haemolyticus	19	8.75						
S.hominis	10	4.60						
S. saprophyticus	10	4.60						
S. sciuri	1	0.45						
S. warneri	23	10.59						
S. xylosus	2	0.90						
Streptococcus parasanguinis	1	0.45						
Streptomyces nogalater	2	0.90						
Total	**217**	**100.0**		**55**	**100.0**		**11**	**100.0**

Legend: n. = number of isolated.

Microbiological analyses of the swabs obtained from the hands of the 131 surgeons after the antisepsis process showed that no microorganism grew from 88 (72.5%) of the samples, but there was growth of 49 colonies in samples from the hands of 36 (27.5%) surgeons. (Table 2).

The identification carried out by MALDI-TOF showed 27 different species of microorganisms with the presence of mixed infections in 8 (22.2%) of these samples, where it was possible to verify the predominance of gram-positive microorganisms on the hands of these surgeons after surgical antisepsis (Table 2). In addition, some fungal species were identified before and after the antisepsis process, including *Candida parapsilosis*, *Aspegillus versicolor*, and *Trichosporon asahii* (Tables 1 and 2).

Table 2. Distribution of the frequency of 49 species isolated from the hands of 36 surgeons who presented contamination after the surgical antisepsis process.

species	type	N.	%	mixed contamination	N.	%
Acinetobacter baumanii	gram-neg.	1	2.0	*S. warneri, M. luteus, K. kirstinae*	1	2.0
Pantoea septica	gram-neg.	1	2.0	*S. warneri, A. baumanii, M. luteus*	1	2.0
Pseudomonas aeruginosa	gram-neg.	1	2.0	*T. asahii. B. lata, M. luteus*	1	2.0
Pseudomonas gessardi	gram-neg.	1	2.0	*S. cohnii, M. luteus*	1	2.0
Serratia marcescens	gram-neg.	1	2.0	*S. xylosus, S. conhnii*	1	2.0
*Burkholderia lata**	gram-neg.	1	2.0	*A.versicolor, C. parapsolosis*	1	2.0
Stenotrophomonas maltophilia	gram-neg.	2	4.0	*S. capitis, M. luteus*	1	4.0
	subtotal	**8**	**16.3**	*S. warneri, S. saprophyticus*	1	2.0
				subtotal	**8**	**16.3**
Bacillus cereus	gram-pos.	2	4.0			
Bacillus simplex	gram-pos.	1	2.0			
Brevibacterium ravenspurgense	gram-pos.	1	2.0			
Dermacoccus nishinomyaensis	gram-pos.	1	2.0			
Kokuria kirstinae *	gram-pos.	1	2.0			
Micrococcus luteus *	gram-pos.	6	12.2			
Staphylococcus warneri *	gram-pos.	7	14.2			
Staphylococcus aureus	gram-pos.	1	2.0			
Staphylococcus capitis *	gram-pos.	6	12.2			
Staphylococcus caprae	gram-pos.	1	2.0			
Staphylococcus cohnii *	gram-pos.	3	8.2			
Staphylococcus epidermidis	gram-pos.	2	4.0			
Staphylococcus haemolyticus	gram-pos.	1	2.0			

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Staphylococcus hominis	gram-pos.	2	4.0		
Staphylococcus saprophyticus *	gram-pos.	1	2.0		
Staphylococcus xylosus *	gram-pos.	1	2.0		
	subtotal	**37**	**75.5**		
Aspegillus versicolor *	fungus	1	2.0		
Candida parapsilosis *	fungus	2	4.0		
Trichosporon asahii *	fungus	1	2.0		
	subtotal	**4**	**8.2**		
	Total	**49**	**100**	**8**	**100**

Legend: Neg = negative. Pos = positive.

3.2. Antimicrobial susceptibility tests

The results of antimicrobial susceptibility tests for gram-positive microorganisms isolated from the hands of physicians after surgical antisepsis obtained by the BD Phoenix™ automated system (AST-Pmic89) showed sensitivity for all isolates evaluated, and only *S. aureus* presented an intermediate profile of sensitivity to levofloxacin. The results of the automated system Vitek2 (AST-N408) for the susceptibility profile for glucose-fermenting and nonfermenting gram-negative microorganisms showed that *Pseudomonas aeruginosa* was resistant to the carbapenems imipenem and meropenem; *P. gessardi* was resistant to aztreonam; and *Serratia marcescens* was resistant to ampicillin. The other results are shown in Table 3.

It is important to note that no growth of microorganisms was observed in any of the 12 water samples collected from the two lavatories that were used for handwashing before surgical procedures.

Table 3. Antibiotic resistance profiles obtained by the automated Vitek2 system for gram-negative non-fermenting bacilli and glucose-fermenting bacilli isolated from surgeons' hands after an antisepsis process.

	non-glucose fermenting bacilli						**glucose-fermenting bacilli**
microorganisms / **antibiotics**	***Stenotrophomonas maltophilia* n=2**	***Acinetobacter baumannii* n=1**	***Pseudomonas aeruginosa* n=1**	***Pseudomonas gessardi* n=1**	***Burkholderia lata* n=1**	***Pantoea septica* n=1**	***Serratia marcescens* n=1**
amikacin	-	Sus.	Sus.	Sus.	Na	Sus.	Sus.
ampicillin	-	-	-	-	-	-	Res.
aztreonam	-	-	-	Res.	Na	-	-
cefepime	-	-	Int.	-	Na	Sus.	Sus.
ceftazidime	-	-	Int.	Int.	Na	-	Sus.
ceftazidime-avibactam	-	-	-	Sus.	Na	-	-
ceftriaxone	-	-	-	-	Na	Sus.	Sus.
ciprofloxacin	-	Sus.	Int.	Int.	Na	Sus.	Sus.
gentamicin	-	Sus.	-	-	Na	Sus.	Sus.
imipenem	-	Sus.	Res.	Int.	Na	Sus.	Sus.
meropenem	-	Sus.	Res.	Sus.	Na	Sus.	-
piperacillin-tazobactam	-	-	Int.	Int.	Na	-	Sus.
Levofloxacin	Sus.	Int.	Int.	Int.	Na	Sus.	-
Sulfamethoxazole + trimethoprim	Int.	Int.	-	-	Na	Sus.	-

Legend: Na= Not applicable; Sus= susceptible; Int= Intermediary; Res= Resistan.

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3.3. Statistical analysis

Statistical analysis of the frequency distribution of the sociodemographic variables of the 131 surgeons analyzed showed that 108 (82.4%) were men and 23 (17.5%) were women.

The McNemar test for the presence and absence of microorganisms on the surgeons' hands before and after surgical antisepsis showed a very high frequency (100%) before washing, and after antisepsis, this frequency dropped significantly ($p<0.05$) to 27.5%. The Mann–Whitney test of numerical variables in relation to the surgeon's gender showed that the surgeon's gender had a significant influence ($p<0.05$) only on the physician's age; on average, the women were younger than the men. Sex had no influence on the other numerical variables.

Concerning age, time since graduation, specialties, and titration, the results are shown in Table 4. The age groups with the highest number of surgeons were from 30 to 39 years (32.8%) and 40-49 years (22.9%). Most of them, 49 (37.4%), were between 1 and 5 years after graduation; only 14 (10.7%) were more than 30 years post-graduation. As for the specialty, the two most prevalent were general surgery (42, 32.1%) and orthopedics (27, 20.6%). Regarding their degrees, 73 (55.7%) were specialists, and only 7 (5.3%) had a doctorate (Table 4).

Table 4. Frequency distribution of socio-demographic variables among the 131 interviewed surgeons**.**

variables		number	percentage (%)
age group	< 30	27	20.6
	30-39	43	32.8
	40-49	30	22.9
	50-59	20	15.3
	60-69	8	6.1
	70 ou +	3	2.3
time since graduation (years)	1 a 5	49	37.4
	6 a 10	25	19.1
	11 a 15	13	9.9
	16 a 20	9	6.9
	21 a 25	6	4.6
	26 a 30	15	11.4
	> 30	14	10.7
specialties	General surgery	42	32.1
	Orthopedics	27	20.6
	Urology	11	8.4
	Vascular surgery	9	6.9
	Digestive system	8	6.1
	Cardiac surgery	7	5.3
	Coloproctology	6	4.6
	Neurosurgery	6	4.6
	Head neck	4	3
	Maxillary mouth	3	2.3
	Plastic surgery	3	2.3
	Otorhinolaryngology	3	2.3
	Thoracic Specialties surgery	2	1.5
titration	Resident	39	29.8
	Specialist	73	55.7
	Master	12	9.2
	Doutor	7	5.3

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Data from clinical variables on how to perform the surgical antisepsis process (brushing time, sequence, products used), knowledge of international safety goals, risk of infection in surgery, safe surgery protocol, and occurrence of surgical site infection (ISC) are presented in Table 5. The results obtained showed that 100 (76.3%) of the surgeons spent 3 to 5 min in the brushing process, 73 (55.7%) performed the technique correctly, 104 (79.4%) used chlorhexidine, and 91 (69.5%) safe surgery protocol (Table 5).

Of the 100 surgeries performed by the 131 physicians after antisepsis of the hands, laparoscopic cholecystectomy was the most common surgical procedure, accounting for 15 (11.4%) (Table 6).

Table 5. Frequency distribution of clinical variables in surgical hand antisepsis by surgeons and safety goals.

variables		n	%
brushing time (minutes)	2	25	19.1
	3 to 5	100	76.3
	> 5	6	4.6
sequence	Correct	73	55.7
	Incorrect	58	44.3
product	Water plus povidone	3	2.3
	Chlorhexidine	104	79.4
	Povidone iodine	24	18.3
risk of infection in surgery	Contaminated	8	6.1
	Infected	7	5.3
	Clean	91	69.5
	Potentially contaminated	25	19.1
knows the six international patient safety goals	In part	83	63.3
	No	30	23
	Yes	18	13.7
what is the goal *	Ignore	45	34.1
	1- Correctly identify the patient	70	53
	2- Improve communication between health professionals	6	4.5
	3- Improve safety in the prescription, use and administration of medications	9	6.8
	4- Ensure surgery in the correct intervention site, procedure, and patient	88	66.7
	5- Sanitize your hands to avoid infections	26	19.7
	6- Reduce the risk of falls and pressure ulcers	11	8.3
responds to safe surgery protocol	No	31	23.7
	Yes	100	76.3
cirurgical site infection (SSI)	Ignore	15	11.4
	No	91	69.5
	Yes	25	19.1

Legend. * = multiple answers.

Table 6. Frequency distribution of types of procedures performed after antisepsis of hands.

type of surgery	n	%	type of surgery	n	%
laparoscopic cholecystectomy	15	11.4	thyroidectomy	2	1.5
tibial osteosynthesis	7	5.3	wrist fracture	2	1.5
inguinal hernioplasty	6	5.3	valve replacement	2	1.5
appendectomy	6	3.8	abdominoplasty	1	0.8

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prostatectomy	5		hand amputation	1	0.8
discectomy	4	3	brain aneurysm	1	0.8
carotid endarterectomy	4	3	maxillary antrostomy	1	0.8
laparotomy	4	3	spine arthrodesis	1	0.8
myocardial revascularization	4	3	hip arthroplasty	1	0.8
thigh amputation	3	2.3	knee arthroscopy	1	0.8
gastroplasty	3	2.3	thyroglossal cyst	1	0.8
hemorrhoidectomy	3	2.3	colectomy	1	0.8
femur osteosynthesis	3	2.3	pancreaticoduodenectomy	1	0.8
intestinal transit reconstruction	3	2.3	chest tumor excision	1	0.8
bone tumor resection	3	2.3	fasciotomy	1	0.8
tracheostomy	3	2.3	arteriovenous fistula	1	0.8
choledochotomy	2	1.5	cerebrospinal fluid fistula	1	0.8
hand debridement	2	1.5	mandibular fracture	1	0.8
leg debridement	2	1.5	hepatectomy	1	0.8
excision of saliva calculus	2	1.5	incisional hernia	1	0.8
brain tumor excision	2	1.5	lymphadenectomy	1	0.8
open fracture leg	2	1.5	mammaplasty	1	0.8
gastrectomy	2	1.5	mastectomy	1	0.8
hysterectomy	2	1.5	nephrectomy	1	0.8
shoulder arthroscopy	2	1.5	radius osteosynthesis	1	0.8
laparoscopic nephrectomy	2	1.5	transurethral resection of the prostate	1	0.8
wrist osteosynthesis	2	1.5	thoracoscopy	1	0.8
sinusotomy	2	1.5	urethroplasty	1	0.8

4. Discussion

In the present study, the results of the evaluation of the microbiological profile of the hands of resident physicians, specialists, masters, and doctors before and after surgical antisepsis showed that despite the existence of a compulsory protocol for daily use in the researched hospital, the frequency rates of microorganisms on the hands of the evaluated surgeons were very high at both timepoints: before handwashing, (100%) and after this procedure (27.5% of the doctors still preserved microorganisms in their hands).

The importance of hand antisepsis for patient safety is not a new concept. Ignaz Philip Semmelweis, in 1847, established an important correlation between medical care and a higher maternal risk of puerperal fever since the rates were much lower when parturients were assisted by midwives [28].

The high prevalence of gram-positive bacteria found before and after the antisepsis procedure in the evaluated professionals' hands, especially by species of the *Staphylococci* genus, can theoretically be explained, as these are part of the microbiota of the human skin and mucosa [29]; however, their permanence in the hands of surgeons after antisepsis is a worrying fact because these microorganisms are important opportunistic pathogens that have shown high rates of involvement in infections in hospitalized patients [30,31].

The presence of different species of microorganisms, such as Staphylococcus warneri, Staphylococcus capitis, Staphylococcus hominis, Staphylococcus hemolyticus, Micrococcus luteus, and Stenotrophomonas maltophilia, on the hands of surgeons after antisepsis calls attention to one more complicating factor for the hospital unit in question, and it is worth noting that 8 professionals from the same team of doctors work at another public hospital in São Luis-MA. Szemraj et al. [32] emphasize the importance of coagulase-negative staphylococci, including S. hemolyticus, S. hominis, S. warneri, and S. simulans, as etiological agents of serious infections.

The hospital environment is especially favorable to the circulation of numerous species of microorganisms, pathogenic or not, as it has its own characteristics, including the high flow of sick

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people, companions, visitors, cleaning teams, kitchen staff, nurses, doctors, and laboratory workers, among other groups [33]. In addition, there are factors related to the constant, and sometimes indiscriminate, use of different classes of antimicrobials, which add to the need for invasive procedures; the use of beds in intensive care units; the use of equipment for diagnostic tests; the microbial load on the surfaces of walls and bedroom furniture, door handles, sinks, toilets, and faucets; in short, there is a highly complex dynamic involved in the operation of a hospital that makes it difficult to pinpoint where outbreaks of infection begin [4,6–8,33].

The findings on the hands of physicians after antisepsis, described in Table 2, should not be ignored, as the presence of several bacterial species being carried by the surgeons themselves to the hospital raises interest in the occurrence of HAI by microorganisms with a potential opportunistic nature. Among these, *Bacillus cereus* stands out; it is a ubiquitous sporulated *bacillus* present in the soil and food that can easily be taken to the hospital environment through the skin and has been implicated in nosocomial infections [34]. *Bacillus simplex* is a microorganism of the environment whose habitat is soil; a recent report documented the occurrence of infection in a traumatic injury [35].

Kokuria kirstinae can colonize the skin, mucous membranes, and oropharynx, having already been associated with invasive infections in immunocompromised patients, especially children [36–39]; *Brevibacterium ravenspurgense* may be present on human skin; no reports of involvement of this species in HAIs were found, and its detection in the present study may represent an alert for infection control committees. However, there is a prior report of commensal *B. casei* in a child with acute leukemia [40].

The gram-negative bacillus *Stenotrophomonas maltophilia* can be found in plants, in soil, and on the surface of human skin. Cases of infections in the respiratory and intestinal tracts have been described in immunocompromised patients with malignant tumors, diabetes, and the use of immunosuppressants [41]. In addition, large outbreaks of infections caused by *Acinetobacter baumannii* and *Pseudomonas aeruginosa* have been documented and implicated in HAIs worldwide, as these bacteria are considered opportunistic pathogens that carry genes that confer multiresistance profiles to several classes of antimicrobials [42].

Interestingly, the isolates recovered from surgeons' hands after an antisepsis process, such as gram-negative nonfermenting bacilli *P. aeruginosa* and *P. gessardi*, showed profiles of resistance to carbapenems, imipenem and meropenem, and to aztreonam, respectively. However, the glucose-fermenting bacilli *Serratia marcescens* was resistant to ampicillin. Among the species of the genus *Pseudomonas, P. aeruginosa* is considered an important cause of HAIs and is one of the major resistant gram-negative pathogens [17].

Candida species, which emerged as opportunistic pathogens, including *C. parapsilosis* and other fungi such as *Aspegillus versicolor, Rhodothorula mucilaginous* and *Trichosporon asahii*, are involved in invasive infections in immunocompromised patients, mainly those with cancer, undergoing organ transplants, or receiving immunosuppressant drugs after transplantation pathogens [43].

The analysis of the sociodemographic data of the evaluated surgeons showed a low number of physicians with a doctorate; most of them were between 30 and 39 years old, and they had a short time since graduation, from 1 to 5 years. In view of the above, it is noteworthy that, naturally, surgeons use sterile gloves, but if some are not able to perform the correct antisepsis of the hands when putting on the gloves, they could contaminate the gloves during this process and, in this case, become a source of infection for their patient during the surgical procedure.

5. Conclusions

The results of this study showed that the surgeons of the researched hospital use the handwashing protocol inefficiently because there is a high frequency of microorganisms after the antisepsis process. Hand hygiene practices should be reinforced, and in this scenario, greater adherence to strict protocols is needed through the adoption of educational strategies and policies aimed at surveillance, management and control of HAIs, mainly surgical site infection if barriers such as gloves are punctured.

Author Contributions: All authors contributed to the article. A.S.N. prepared the research project, collected the samples, applied the questionnaire to obtain the sociodemographic data of the surgeons studied, and helped write the manuscript. S.G.M. isolated the microorganisms, performed the automated identification and antimicrobial susceptibility tests, and analyzed the results. M.R.Q.B. analyzed the results, prepared the tables, and wrote the manuscript. S.G.M. tabulated the data, interpreted the data, and performed the statistical analysis of the results. R.C.d.S. co-supervised the project from its conception to the final results, and helped in the preparation of the manuscript. R.A.N. supervised the writing and execution of the project from its conception to the final results, and helped in the preparation of the manuscript. All authors have read and agreed to the published version of the manuscript.

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References

1. Haque, M.; Sartelli, M.; McKimm, J.; Abu Bakar, M. Healthcare-associated infections - an overview. *Infect Drug Resist* 2018, 11, 2321-2333. https://doi: 10.2147/IDR.S177247.
2. Pinto, H.; Simões, M.; Borges, A. Prevalence and Impact of Biofilms on Bloodstream and Urinary Tract Infections: A Systematic Review and Meta-Analysis. *Antibiotics* 2021,10, 825. https://doi: 10.3390/antibiotics10070825.
3. Allegranzi, B.; Nejad, S.B, Pittet, D. The Burden of Healthcare--Associated Infection."Hand hygiene: a handbook for medical professionals". *Wiley-Blackwell* 2017, 1-7**.** https://doi.org/10.1002/9781118846810.ch1.
4. Vermeil, T.; Peters, A.; Kilpatrick, C.; Pires, D.; Allegranzi, B.; Pittet, D. Hand hygiene in hospitals: anatomy of a revolution. *J Hosp Infect* 2019, 101, 4:383-392. https://doi: 10.1016/j.jhin.2018.09.003.
5. Puro, V.; Coppola, N.; Frasca, A.; Gentile, I.; Luzzaro, F.; Peghetti, A.; Sganga, G. Pillars for prevention and control of healthcare-associated infections: an Italian expert opinion statement. *Antimicrob Resist Infect Control* 2022, 11, 87. https://doi.org/10.1186/s13756-022-01125-8.
6. Lotfinejad, N.; Peters, A.; Tartari, E.; Fankhauser-Rodriguez, C.; Pires, D.; Pittet, D. Hand hygiene in health care: 20 years of ongoing advances and perspectives. *The Lancet infect dis 2021, 21*, 8. e209-e221. https://doi.org/10.1016/S1473-3099(21)00383-2.
7. Xavier, HMD.; Silva, G.L.V.; Röder, D.V.D.B. Hands of Health Care Professionals as a Vehicle for the Transmission of Hospital Pathogens. *Acta Scientific Paediatrics* 2021, 4.2, 41-46. https://10.1016/j.jhin.2009.04.019.
8. Sydnor, E.R.; Perl, T.M. Hospital epidemiology and infection control in acute-care settings. *Clin Microbiol Ver* 2011, 1,141-73. https://doi: 10.1128/CMR.00027-10.
9. Monegro, A.F.; Muppidi, V.; Regunath, H. Hospital Acquired Infections. In: StatPearls [Internet]. *Treasure Island 2023.* (FL): StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK441857/.
10. Curtis, L.T. Prevention of hospital-acquired infections: review of non-pharmacological interventions. *J Hosp Infect 2008,* 69.3, 204-19. https://doi: 10.1016/j.jhin.2008.03.018.
11. Guggenbichler, J.P.; Assadian, O.; Boeswald, M.; Kramer, A. Incidence and clinical implication of nosocomial infections associated with implantable biomaterials - catheters, ventilator-associated pneumonia, urinary tract infections. *GMS Krankenhhyg Interdiszip* 2011, 6,1. https://doi: 10.3205/dgkh000175.
12. Sikora, A.; Zahra, F. Nosocomial Infections. [Updated 2023 Apr 27]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK559312/.
13. Chia, P.; Sengupta, S.; Kukreja, A.; Ponnampalavanar, S.S.L.; Oon, T. N.G.; Marimuthu, K. The role of hospital environment in transmissions of multidrug-resistant gram-negative organisms. *Antimicrob Resist Infect Control* 2020, 9,29. https://doi.org/10.1186/s13756-020-0685-1.
14. Sood, G.; Perl, T.M. Outbreaks in Health Care Settings. *Infect Dis Clin North Am* 2016, 30.3,661-87. https://doi: 10.1016/j.idc.2016.04.003.
15. Novosad, S.A.; Fike L.; Dudeck M.A.; Allen-Bridson, K.; Edwards, J.R.; Edens, C.; Sinkowitz-Cochran, R.; Powell, K.; Kuhar, D. Pathogens causing central-line-associated bloodstream infections in acute-care hospitals-United States, 2011-2017. *Infect Control Hosp Epidemiol* 2020, 41.3:313-319. https://doi: 10.1017/ice.2019.303.

16. Gall, E.; Long, A.; Hall, K.K. Infections Due to Other Multidrug-Resistant Organisms. In: Hall, K.K.; Shoemaker-Hunt, S.; Hoffman, L. et al. Making Healthcare Safer III: A Critical Analysis of Existing and Emerging Patient Safety Practices [Internet]. Rockville (MD): *Agency for Healthcare Research and Quality* (US), 2020. https://www.ncbi.nlm.nih.gov/books/NBK555533/.
17. Szabó, S.; Feier, B.; Capatina, D.; Tertis, M.; Cristea, C.; Popa, A. An Overview of Healthcare Associated Infections and Their Detection Methods Caused by Pathogen Bacteria in Romania and Europe. *J Clin Med* 2022,11,3204. https://doi: 10.3390/jcm1111320.
18. Ataee, R.A.; Ataee.; M.H.; Tavana, A.M.; Salesi, M. Bacteriological Aspects of Hand Washing: A Key for Health Promotion and Infections Control. *Int J Prev Med* 2017, 10. 8-16. doi: https://10.4103/2008-7802.201923.
19. Berg, G.; Rybakova, D.; Fischer, D.; Cernava, T.; Verges, M.C.C.; Chen, X.; Cocolin, L.; Eversole, K.; Corral, G.H.; Kazou, M.; Kinkel, L.; Lange, L.; Lima, N.; Loy, A.; Macklin, J.A.; Manguin, E.; Mauchline, T.; McClure, R.; Mitter, B.; Ryan, M.; Sarand, I.; Smidt, H.; Schelkle, B.; Roume, H.; Kiran, G.S.; Selvin, J.; Souza, R.S.C.; Overbeek, L.van.; Singh, B.K.; Wagner, M.; Walsh, A.; Sessitsch, A.; Schloter, M. Microbiome definition re-visited: old concepts and new challenges. *Microbiome 2020,* 8, 103. https://doi.org/10.1186/s40168-020-00875-0.
20. Suleyman, G.; Alangaden, G.; Bardossy, A.C. The Role of Environmental Contamination in the Transmission of Nosocomial Pathogens and Healthcare-Associated Infections. *Curr Infect Dis Rep* 2018, 20,12. https://doi.org/10.1007/s11908-018-0620-2.
21. Mody, L.; Washer, L.L.; Kaye, K.S.; Gibson, K.; Saint, S.; Reyes, K.; Cassone, M.; Mantey, J.; Cao, J.; Altamimi, S.; Perri, M.; Sax, H.; Chopra, V.; Zervos, M. Multidrug-resistant Organisms in Hospitals: What Is on Patient Hands and in Their Rooms? *Clin Infect Dis* 2019, 69.11, 1837-1844. https://doi: 10.1093/cid/ciz092.
22. Mouajou, V.; Adams, K.; DeLisle, G.; Quach, C. Hand hygiene compliance in the prevention of hospital-acquired infections: a systematic review. *J Hosp Infect* 2022,119,33-48. https://doi: 10.1016/j.jhin.2021.09.016.
23. Lima, M.R.P.; Filho, A.O.F.; Bem, J.S.P.; Simões, M.S.S.; Santos, E, F.; Arreguy, I.M.S.; Sobrinho, C.R.W.; Souza, F.B. (2019) Surgical hand preparation without rinsing: influence of antiseptic agent on bacteriological contamination. *J Dent Health Oral Disord Ther* 2019. 10.1, 98–101. https://doi.org/10.15406/jdhodt.2019.10.00467.
24. Widmer, A.F.; Rotter, M.; Voss, A.; Nthumba, P.; Allegranzi, B.; Boyce, J.; Pittet, D. Surgical hand preparation: state-of-the-art. *J Hosp Infect* 2010, *74*.2,112-122. https://doi.org/10.1016/j.jhin.2009.06.020.
25. World Health Organization (WHO). Hand Hygiene Technical Reference Manual: To be used by health-care workers, trainers and observers of hand hygiene practices, 2009. Geneva: WHO; p. 1–31.
26. Brazilian Committee for Antimicrobial Sensitivity Tests – BrCAST/*European Committee on Antimicrobial Susceptibility Testing (EUCAST/versão BrCAST)* (2022). Tables of cut-off points for interpretation of CIMs and diameters of halos. BrCAST. Available online: URL https://brcast.org.br/wp-content/uploads/2022/07/Tabela-pontos-de-corte-clinicos-BrCAST-17-10-2019-final-1.pdf
27. IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.
28. Couto, R.C.; Pedrosa, T.M.G.; Cunha, A.F.A.; Amaral, D.B. Infecção hospitalar e outras complicações não-infecciosas da doença. Epidemiologia, Controle e Tratamento. 4 ed. Guanabara Koogan: Rio de Janeiro, 2009; 832p.
29. Boucher, H.W.; Corey, G.R. Epidemiology of methicillin-resistant Staphylococcus aureus. *Clin Infect Dis* 2008, 1.46 Suppl 5, S344-9. https://doi: 10.1086/533590.
30. Temperoni, C.; Caiazzo, L.; Barchiesi, F. (2021) High Prevalence of Antibiotic Resistance among Opportunistic Pathogens Isolated from Patients with COVID-19 under Mechanical Ventilation: Results of a Single-Center Study. *Antibiotics* 2021, 10.9,1080. https://doi: 10.3390/antibiotics10091080.
31. Seidelman, J.L.; Mantyh, C.R.; Anderson, D.J. Surgical Site Infection Prevention: A Review. *JAMA 2023,* 3,244–252. https://doi:10.1001/jama.2022.24075.
32. Szemraj, M.; Lisiecki, P.; Glajzner, P.; Szewczyk, E.M. Vancomycin heteroresistance among methicillin-resistant clinical isolates *S. haemolyticus*, *S. hominis*, *S. simulans*, and *S. warneri*. *Braz J Microbiol* 2023, 1,159-167. https://doi: 10.1007/s42770-022-00870-7.
33. Dalton, K.R.; Rock, C.; Carroll, K.C.; Davis, M.F. One Health in hospitals: how understanding the dynamics of people, animals, and the hospital built-environment can be used to better inform interventions for antimicrobial-resistant gram-positive infections. *Antimicrob Resist Infect Control* 2020, 9,78. https://doi.org/10.1186/s13756-020-00737-2
34. Glasset, B.; Herbin, S.; Granier, S.A.; Cavalié, L.; Lafeuille, E.; Guérin, C.; Ruimy, R.; Casagrande-Magne, F.; Levast, M.; Chautemps, N.; Decousser, J.W.; Belotti, L.; Pelloux, I.; Robert, J.; Brisabois, A.; Ramarao, N. *Bacillus cereus*, a serious cause of nosocomial infections: Epidemiologic and genetic survey. *PLoS One* 2018, 13,5. e0194346. https:// doi: 10.1371/journal.pone.0194346.
35. Xaplanteri, P.; Serpanos, D.S.; Dorva, E.; Beqo-Rokaj, T.; Papadogeorgaki, E.; Lekkou, A. *Bacillus simplex* as the Most Probable Culprit of Penetrating Trauma Infection: A Case Report. *Pathogens* 2022, 211.10,1203. https://doi: 10.3390/pathogens11101203.

 doi:10.20944/preprints202306.0776.v1

36. Chen, H.M.; Chi, H.; Chiu, N.C.; Huang, F.Y. *Kocuria kristinae*: A true pathogen in pediatric patients. *J Microbiol Immunol Infect* 2015, 48,80–84. https://doi: 10.1016/j.jmii.2013.07.001.
37. Robles-Marhuenda, A.; Romero-Gomez, M.P.; Garcia-Rodrıguez, J.; Arnalich-Fernandez, F. Native valve endocarditis caused by Kocuria kristinae. *Enferm Infecc Microbiol Clin* 2016, 34, 464–465. https://doi: 10.1016/j.eimc.2015.09.008
38. Gunaseelan, P.; Suresh, G.; Raghavan, V.; Varadarajan, S. Native valve endocarditis caused by *Kocuria rosea* complicated by peripheral mycotic aneurysm in an elderly host. *J Postgrad Med* 2017, 63.2, 135-137. https://doi: 10.4103/jpgm.JPGM_441_16.
39. **Taher,** N.M. *Kocuria* Species: Important Emerging Pathogens in Pediatric Patients. *J Pure Appl Microbiol* 2022, 4,2874-2879. https://doi.org/10.22207/JPAM.16.4.60.
40. Bal, Z.S.; Sem, S.; Karapinar, D.Y.; Aydemir, S.; Vardar, F. The first reported catheter-related *Brevibacterium casei* bloodstream infection in a child with acute leucemia and review of the literature. *Braz. J Infect Dis* 2015, 19, 213-215. https://doi: 10.1016/j.bjid.2014.09.011.
41. Liu, B.; Tong, S. An investigation of *Stenotrophomonas maltophilia*-positive culture caused by fiberoptic bronchoscope contamination. BMC Infect Dis 2019, 19.1,1072. https://doi: 10.1186/s12879-019-4670-3.
42. Wieland K.; Chhatwal P.; Vonberg, R.P. Nosocomial outbreaks caused by *Acinetobacter baumannii* and *Pseudomonas aeruginosa:* Results of a systematic review, *Am J Infect Cont* 2018, 6, 643-648. https://doi.org/10.1016/j.ajic.2017.12.014.
43. Xia, J.; Wang, Z.; Li, T.; Lu, F.; Sheng, D.; Huang, W. Immunosuppressed Patients with Clinically Diagnosed Invasive Fungal Infections: The Fungal Species Distribution, Antifungal Sensitivity and Associated Risk Factors in a Tertiary Hospital of Anhui Province. Infect Drug Resist 2022, 15,321-333. https://doi: 10.2147/IDR.S351260.