Incidence of multiresistant antimicrobial *Staphylococcus* in the hands of Health Basic Unit professionals

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Abstract

Staphylococcus is an important etiologic agent of infections in hospital and healthcare settings. One of the means of proliferation of these agents is the contamination of the hands of professionals who perform health care. This research aimed to identify the possible incidence of multiresistant Staphylococcus aureus colonized in the hands of health professionals, before and after hand hygiene, and its correlation with the spread of infections in these environments. The research was carried out by biochemical tests in the laboratory, of which results indicated the colonization by Staphylococcus even after hand hygiene (88.3% positive and 11.7% negative); this allows the dissemination of the bacteria to other patients and environments. The samples were submitted to the multiple antimicrobial resistance index, and data showed the persistence of microorganisms resistant to the 15 antimicrobials used. So additional studies need to be done in order to suggest effective actions and awareness of health professionals about hand hygiene as an important preventive action in hospital and health care settings.

Keywords: *Staphylococcus*; Infections, Hand disinfection; Pathogenic bioagents; Human microbiota; Antibiotics.

1. Introduction

Health care workers are constantly exposed to pathogenic bioagents in their different jobs, but most of the time they do not get sick. This does not indicate that health care workers are more resistant, but because the normal microbiome is intrinsically resistant to colonization and infection by external microorganisms. A number of factors, including hand hygiene, the range of their exposure to contagious agents, modifies this intrinsic resistance and the inherent immunocompetence associated with the ecological relationships between the pathogen and the host microbiome[1].

The human microbiome is associated with host health and disease, but most of the evidence supporting this association is based on studies of intestine microorganisms. For the microbiome of other body parts, including the skin, it is not clear to what extent it influences a person's ability to carry or resist a pathogen[2,3,4]. However, there is evidence that health care workers' hands are the most common transmission vector of microorganisms from patient to patient, as well as within the hospital environment[5,6]. Human skin is permanently colonized by physiological or resident microbiome and, temporarily, depending on nature and its characteristics by several pathogenic agents that belong to the transient microbiome[7,8]. The microorganisms of the resident microbiome are found mainly on the skin surface and under the superficial cells of the stratum corneum, and are not considered pathogenic in intact skin, but can cause infections in sterile body cavities, in the eyes, or the skin with the presence of lesions[9].

It is important to highlight that the transitory microbiome is composed of microorganisms that colonize the more superficial layers of the skin, considered potential infectious pathogens[10]. It can be acquired from the hospital environment, by direct contact between professionals and patients, by contact with contaminated surfaces or objects, or as a consequence of poor hygiene, thus being responsible for cross-infection[8,11]. The predominance of gram-negative bacteria, especially Enterobacteriaceae, bacteria type *Pseudomonas* and *Acinetobacter*, gram-positive bacteria of *Staphylococcus* and spore-forming anaerobic, and, fungi and viruses, with higher pathogenicity, are commonly associated with outbreaks of severe nosocomial infection due to their high and multiple antimicrobial resistance patterns[9,10,12]. About 20% of individuals are carriers of *Staphylococcus*, called persistent carriers; however, approximately 60% are considered intermittent carriers, and the others never show up as colonized[13].

The species *Staphylococcus aureus* has shown to be an important etiological agent of nosocomial infections with the ability to acquire resistance to antimicrobials. The Intensive Care Units (ICU) stand out as the place of the predominance of such microorganisms associated with the most relevant adverse events related to the care of patients[14-17]. In synthesis, professionals working in ICUs and internal medical rooms are more prone to contamination. This can be explained by the more frequent contact with patients and other unidentified factors[15].

Hand hygiene has been identified as one of the most effective procedures to control the transmission of infections in hospital environments, and educating professionals about it is an important tool to ensure its successful implementation. To persuade users, and as part of education, it is important to provide evidence on the importance of hand hygiene in reducing bacterial microbiota[11,18-20]. Evidence-based hand hygiene can prevent the transmission of nosocomial pathogens and, also, keep the health care team's skin healthy. In most clinical scenarios, antisepsis is recommended for hand decontamination for better efficacy and cutaneous tolerance. Whereas conformity could be improved by the knowledge of the main clinical circumstances, in which hand antisepsis by health care professionals really benefits the patient[8].

Due to the patient's safety barrier failures and prevention control errors concerning hand hygiene, the present objective of the study was to verify the incidence of multidrug-resistant *Staphylococcus* antimicrobials in the hands of the Basic Health Unit (BHU) professionals.

2. Materials and methods

Sixty professionals from BHU Fernandópolis/SP collaborated in this study, being nurses, technicians and nursing assistants, resident doctors, pharmacists, pharmacy and medicine interns, clerks, dentists, X-Ray technicians, of both genders. The Research Ethics Committee of the Camilo Castelo Branco University under the ruling 1.560.268 and CAAE number 54979316.4.0000.5494 approved the study. Professionals who agreed to participate and signed the Free and Informed Consent Term were included in the study. Professionals who were in skin treatment were excluded.

The samples were collected with the help of a sterile Swab before and after the participants' hands were sanitized. The Swab was rubbed on the hand palm and the inter-digitals in zigzag, in one direction, deposited in a sterile test tube and taken to the microbiology laboratory for dilutions. This technique followed the methodology described by Alwis et al[6]. For hand hygiene, it was used soap and water for a period of thirty seconds, following the BHU routine. The study was carried out in triplicate.

The samples were submitted to serial dilutions in 0.5% NaCl solution for culture in Baird Parker Agar environment, incubated at 37ºC for 24-48 hours. Colonies were counted and results were expressed in Colony Forming Units (CFU).

Typical *Staphylococcus* colonies were submitted to Gram staining and observed under a light microscope. Once the morphological characteristics were confirmed, these colonies were sub cultivated in the Baird Parker Agar environment and submitted to biochemical catalase and coagulase tests[21].

The identified *Staphylococcus* were evaluated regarding the susceptibility profile to antimicrobials. The disk diffusion method was used, and the antimicrobials evaluated were: amoxicillin (AMC) 30µg, Ampicillin (AMP) 10µg, Cephalothin (CPL) 30µg, Ciprofloxacin (CIP) 5µg, Clindamycin (CLI) 2µg, chloramphenicol (CLO) 30µg, erythromycin (ERY) 15µg, gentamicin (GEN) 10µg, oxacillin (OXA) 1µg, cefoxitin (CFO) 30µg, penicillin G (PEN) 10µg, rifampicin (RIF) 5µg, sulfazotrim (SUT) 25µg, tetracycline (TET) 30µg, and vancomycin (VAN) 30µg. The results were interpreted in accordance with the parameters established by the Clinical and Laboratory Standards Institute (CLSI)[22].

The multiple antimicrobials resistance (MAR) index was calculated according to the methodology described by Krumperman[23]. It was calculated by the ratio between the numbers of antibiotics to which the isolated one showed resistance and the number of antibiotics to which the isolated one was exposed, MAR greater than 0.2 showed multidrug resistance.

For statistical analysis, Fisher's exact test and tests for one and two proportions were applied to observe significant differences between frequencies; Wilcoxon's test to observe significant differences between the *Staphylococcus* count before and after sanitization; and Kruskal-Wallis test to compare antibiogram results. All statistical tests were performed at a 5% or ($P<0.05$) significance level, and the *software* used was Minitab 17 (Minitab Inc.).

3. Results

Most of the professionals evaluated were female (n=44 - 73.3%), as for employees were pharmaceutical and medicine interns (n=25 - 41.7%), physicians (n=15 - 25.0%), nursing assistants (n=5 -

8.3%), clerks (n=5 - 8.3%), nurses (n=4 - 6.7%), dentists (n=2 - 3.3%), pharmacist (n=2 - 3.3%), X-ray technician (n=1 - 1.7%) and nursing technician (n=1 - 1.7%).

The percentages referring to the *Staphylococcus* presence analysis before and after sanitizing the hands were also analyzed (Table 1), and it was possible to observe that, through the test for two proportions, there was no difference between the presence of *Staphylococcus* before and after health care professionals hand sanitizing since the resulting p-value was higher than the level of significance used for the test.

Table 1. Occurrence percentage of *Staphylococcus* before and after sanitizing the health care professionals' hands assessed in the study.

Staphylococcus	**Before**	**After**	**p[1] Value**
presence	45 (75,0%)	53 (88,3%)	0,097
absence	15 (25,0%)	7 (11,7%)	

[1]p value referring to the Fisher's exact test at p<0,05.

One result that deserves to be highlighted in this analysis was the increased occurrence of *Staphylococcus* in the hands of the health care professionals evaluated after the sanitizing, due to the expectation of opposite result. This outcome can be justified by the fact that 52 (86.7%) professionals evaluated did not sanitize their hands with the correct technique, and only 8 (13.3%) health care professionals sanitized their hands using the appropriate procedure.

The colonies with *Staphylococcus* morphology characteristic, confirmed by Gram staining and light microscopic observation, verified that 66.1% presented positive results for the coagulase enzyme while 33.9% were negative for this test (p=0.015).

Table 2 shows the descriptive statistics of the *Staphylococcus* count on the hands of the 60 professionals evaluated before and after sanitization.

Table 2. Descriptive statistics of *Staphylococcus* count of the health care professionals' hands assessed before and after sanitization.

Sanitization	**Rate ± Pattern Deviation**	**Median**	**(Min; Max)**	**p[1] Value**
before	$2{,}4.10^3 \pm 6{,}3.10^3$	$0{,}4.10^1$	$(0{,}0;4.10^4)$	<0,001
after	$2{,}8.10^3 \pm 3{,}0.10^4$	$0{,}1.10^1$	$(0{,}0;4{,}1.10^5)$	

[1]p value referring to the Wilcoxon test at p<0,05.

The data in Table 2 show that the count of *Staphylococcus* before and after hand sanitation showed significant differences (p<0.001), assuming that the count after sanitation was significantly lower than the count before sanitation. Such a result was possible to be observed due to the median values, since the statistical test applied was non-parametric, precluding the average analysis due to the high pattern deviation and coefficient of variation of data distributions. It was verified that 41 (68.33%) health care professionals presented *Staphylococcus aureus* before and after hand sanitation.

A total of 15 antibiotics were evaluated to observe *Staphylococcus* resistance to these compounds. Results showed that the bacteria multidrug resistance was significant compared to the antibiotics assessed,

since the proportion of resistant bacteria (64.5%) was significantly higher, (p=0.030), than the sensitive bacteria proportion (33.5%).

Table 3 displays the antibiogram descriptive statistics of the bacteria evaluated in relation to antibiotics investigated. Antibiogram results demonstrated that there were no significant differences in the antibiogram of the bacteria when all antibiotics were compared (p=0.091). The high data variation showed the need to use a non-parametric comparison test to support the statistical result. As the p-value was higher than the significance level (p<0.05), there were no significant differences in the comparison of the antibiograms.

Table 3. *Staphylococcus* antibiogram about the antibiotics studied.

Antibiotics	Rate ± Pattern Deviation	Median	(Min; Max)	p[1] Value
AMC	11,17±12,64	3,00	(0,0;32,0)	
SUT	7,65±12,14	0,00	(0,0;35,0)	
CIP	13,22±12,91	14,50	(0,0;35,0)	
CPL	15,65±16,84	5,00	(0,0;44,0)	
GEN	9,85±10,84	0,00	(0,0;27,0)	
AMP	8,76±11,53	0,00	(0,0;39,0)	
OXA	8,39±10,19	0,00	(0,0;33,0)	
CLI	9,46±10,67	0,00	(0,0;30,0)	0,091
VAN	10,33±10,61	10,00	(0,0;30,0)	
CLO	8,02±11,72	0,00	(0,0;35,0)	
RIF	10,35±14,31	0,00	(0,0;40,0)	
ERY	6,61±9,51	0,00	(0,0;30,0)	
PEN	8,43±12,25	0,00	(0,0;33,0)	
TET	5,54±9,72	0,00	(0,0;35,0)	
CFO	9,65±12,57	0,00	(0,0;33,0)	

[1]p value referring to the Kruskal-Wallis test at p<0,05.

Table 4 shows the percentages of bacteria resistance to each of the antibiotics evaluated. The results show that seven antibiotics (SUT, AMP, CLO, ERY, PEN, TET, and CFO) presented proportions that differed significantly when compared between resistant and non-resistant microorganisms. In all cases that this difference was significant, the proportion of resistant microorganisms was significantly higher than the proportion of non-resistant microorganisms.

Table 4. Occurrence percentage of *Staphylococcus* resistance in relation to the antibiotics studied.

Antibiotics	Non-resistant		Resistant		
	N	%	N	%	p Value
AMC	18	39,13	28	60,87	0,184
SUT	13	28,26	33	71,74	0,005
CIP	23	50,00	23	50,00	1,000
CPL	21	45,65	25	54,35	0,659
GEN	21	45,65	25	54,35	0,659
AMP	13	28,26	33	71,74	0,005
OXA	19	41,30	27	58,70	0,302
CLI	18	39,13	28	60,87	0,184
VAN	21	45,65	25	54,35	0,659
CLO	15	32,61	31	67,39	0,026
RIF	17	36,96	29	63,04	0,104
ERY	14	30,43	32	69,57	0,011
PEN	6	13,04	40	89,96	<0,001
TET	10	21,74	36	78,26	<0,001
CFO	13	28,26	33	71,74	0,005

By analyzing the average and median values it was possible to observe that the great majority of the evaluated microorganisms were classified as multidrug-resistant, because, on average, the multidrug resistance index resulted in a value much higher than 0.2 (Figure 1).

Figure 1. Individual values for the multidrug resistance index of the evaluated microorganisms.

4. Discussion

Infection transmission by contaminated hands of health care workers is a common pattern observed in most health care facilities[1-3,8,15,19,24,25]. The failure to ensure proper hand hygiene practices is one of the main causes of infections associated with health care and the spread of multidrug-resistant organisms and has been recognized as an important contributor to outbreaks of infectious diseases by the World Health Organization (WHO). The WHO recognizes that health care professionals washing hands with soap can prevent infection in patients and is the most effective and cheapest way to prevent pathogenic microorganism transmission[11].

The hands of health care workers might be colonized by *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus spp*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Clostridium difficile*, *Candida albicans* among other microorganisms[1,5,9,15,18-20].

S. aureus colony is asymptomatic, which makes it possible to contaminate other parts of the body, particularly the hands, which become vectors of microorganism contamination by contact. Since the presence of *Staphylococcus* is high in a hospital environment, this bacterium is responsible for the majority of infections by contamination, especially through the hands of contaminated professionals and patients/users[15,24]. In this study, the hands of the evaluated health care workers were colonized by *Staphylococcus* (Table 1), however *S. aureus* was isolated in most of the participants, (n=41 - 68.33%).

Considering that the health care professionals' hands represent the main transmission route of nosocomial pathogens and are permanently colonized by the resident microbiota and temporarily, by the transitory microbiome, the survival time of microorganisms in tissues must be considered. *Staphylococcus aureus*, for example, can survive for 120 minutes on the hands and is found in 10% to 78% of professionals, *Pseudomonas spp* from 30-180 minutes being isolated in 1-25% of individuals, while *Escherichia coli* from 60-90 minutes not being known the isolations percentage[8].

After hand sanitization, the inefficiency of the technique used by the participants was verified, since the presence of *Staphylococcus* percentages after hand sanitization of the professionals evaluated in the study showed an increase in the occurrence of the microorganism, in contrast to the expected result. From the professionals evaluated, 11.7% (n=7) do not show isolated from the microorganism, while 88.3% (n=53) with the presence of *Staphylococcus* on the hands (Table 1). This finding may be associated with incorrect hand sanitizing techniques or contamination by the material used in the procedure, as well as the age of the professional, and the work shift, significantly favoring transportation of pathogens[1].

In a study performed by Tselebonis et al.[15] *Staphylococcus spp.* was verified that the prevailing microorganism (60.8%), followed by different Gram-negative pathogens (45.6%). ICU staff had a significant probability of contamination with Gram-negative bacteria (95%), regardless of gender or occupation. These authors observed the presence of *Staphylococcus spp* was associated with the work in the internal medicine ward (95%) and the surgical ward (95%), is prevalent in males versus females (81.3% vs. 54.9%, p = 0.008) and doctors versus nursing staff (76.9% vs. 54.8, p = 0.019). Similar results were obtained by Alwis et al.[6], who found that out of 60 medical students, only 40 (66.7%) said they had washed their hands with soap after using the toilet and that more women (83%) used soap to wash their hands than men (50%). The bacterial load on the hands of both sexes showed an increase after the use of the toilet,

being greater among male students, *Staphylococcus aureus* was isolated from the hands of 21 students from both genders.

The infective dose, or number of CFU for most microorganisms, capable of inducing infection in individuals in the local community and/or hospital, is not yet established. The higher the number of CFU (microbial load), the greater the risk of contamination/infection[10,19]. *Staphylococcus* count before and after hand sanitization showed significant differences ($p<0.001$, Table 2). The participants' hands before sanitizing showed counts ranging from 2.4 to $6.3x10^3$ CFU, while after hand washing there was a significant increase in CFU ($2.8x10^3$-$3.0x10^4$). These results are probably related to inadequate use of procedures or materials contamination[6], or even due to superficial hand layers peeling, which bacteria adhered to the deeper strata of the corneal layers survived, or were transferred from one hand to another[10].

Numerous researches have documented that the number of transient and resident microbiome varies considerably from person to person and is relatively constant[3-5,8,9,13,15,18-20,25]. A higher prevalence of antibiotic-resistant microorganisms in the workers' hands related to patient care compared to non-patients and/or outpatients[1,5,14-16]. In the antimicrobial sensitivity tests, it was verified that the majority of *Staphylococcus* isolated patients presented a pattern of multidrug resistance, considering the MAR test, which considers antibiotic-resistant the isolated ones with an index greater than 0.2[23]. The average and median values showed that the great majority of the evaluated microorganisms were classified as multidrug-resistant, because, on average, the multidrug resistance index resulted in a value much higher than 0.2 (Figure 1). Thus, it was found that seven isolated patients did not present multidrug resistance, and the other isolated ones had indexes greater than 0,2, highlighting that, among these, thirteen isolated patients whose MAR was 1.0.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most common agent associated with nosocomial infections, as well as significant morbidity and mortality. Health care workers act as carriers of MRSA and transmit the disease between individuals[1,9]. Antibiotic-resistant microorganisms directly influence increased morbidity, mortality, hospitalization duration and economic burden in hospitals has been significantly noted with such infections. Health care workers play the main role among the various microorganisms' dissemination routes. Studies conducted to assess the prevalence of MRSA carriers among health care workers have found an estimated 10-40%[15,16,20].

Hand sanitization, as a preventive measure against microorganisms' contamination, is indispensable before and after a hospital or health care procedures, with the correct technique and materials. It acts to reduce the potentially pathogenic bacteria transmission, including those resistant to antimicrobials, and the risk of morbidity and mortality due to these infections, in addition to preventing the microorganism's contamination and the infections proliferation, which can considerably reduce their occurrence[3-5,18,25].

Although some difficulties in adhering to hand sanitizing are recognized, most infection control specialists agree that it is a simple and effective method for preventing bacteria transmission and the infection occurrence outbreaks in health care environments[8,18-20].

5. Conclusion

Health care professionals, when providing the patient's safety, should have greater preventive care in the elimination of the microorganisms located in the hand, wrist, and interdigital skin areas. It is reaffirmed that the adhering to the hand sanitizing practices, simple and with antiseptic friction, besides the routine to be followed in the daily life of the health care team, reduces the contamination risks to the patients, to the professionals themselves in the patient-professional relation and the global costs of health care.

In this regard, it is attributed as a preponderant factor to the training of health professionals, enabling them to understand the contagion risks and proliferation, since they are vectors that carry pathogenic bio agents, due to prevention errors and infection control. It is worthy remembering that the adhesion to the practice of hand sanitization enables, besides complying with the prevention norms established for health care, it offers protection and safety to the patient and the professional, extended to the community that, ultimately, may become a repository of these microorganisms when they spread in the environment.

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DECLARATION OF POTENTIAL CONFLICT OF INTEREST

The authors declare no conflict of interest.

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