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Original Article

The impact of an effective 3-step hand hygiene technique in reducing potentially pathogenic microorganisms found on nursing professionals' hands

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Abstract

Introduction: Healthcare-associated infections are concerning adverse events and hand hygiene is considered an essential preventive measure. The objective of the present study was to assess the effect of a correct 3-step hand hygiene technique on reducing of potentially pathogenic microorganisms on hands related to the WHO five moments for hand hygiene.

Methodology: A cross-sectional study was performed by means of direct observation involving 60 Intensive Care Units (ICU) and clinical nursing professionals in a Brazilian hospital. Observations were performed in order to ascertain the adherence rate and the correct technique during health assistance. Additionally, microbiological analysis of material collected from the nursing professional's hands was carried out. Exploratory and inferential analyses were performed on R software and binomial analysis was carried out by using the Z-test. The study was approved by the research ethics committee and covered all the legal principles for the protection of human subjects.

Results: Hand hygiene adherence rate was 63.3%. However, only 13.3% of the professionals performed the correct 3-step hand hygiene technique regarding steps and time. Sixty-five microorganisms were isolated, among which 56.9% were coagulase-negative *Staphylococcus*, 26.2% were Gram-negative bacilli, 7.7% were *Enterococcus faecalis*, and 6.2% were *Candida parapsilosis*. There was no presence of potentially pathogenic microorganisms on the nursing professional's hands who performed the correct three-step technique.

Conclusions: Overall correct hand hygiene technique was poor. The results indicated the presence of potentially pathogenic microorganisms at moments in which hand hygiene was mandatory but was not executed or was executed incorrectly. The 3-step hand hygiene technique proved to be effective when correctly performed since there were no microorganism growth. Larger studies are needed to test if these results can be replicated at a larger scale, since streamlining hand hygiene technique yielded encouraging results.

Key words: Hand hygiene; technique; patient safety; infection control; healthcare-associated infections; nursing team..

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Introduction

Healthcare-associated infections (HAIs) are the most frequent and worrying adverse events among those that have direct consequences on patient safety and, consequently, on the quality of health services, showing high morbidity and mortality [1]. This adverse event has a considerable and largely avoidable economic impact, which includes high direct costs for patients and social costs [2].

Hand hygiene (HH) is considered an essential measure for preventing HAIs and dissemination of antimicrobial resistance [2]. Evidence has suggested that compliance to HH practices substantially lowers the rates of acquisition of pathogens on the hands, which results in decreased dissemination of these microorganisms in the hospital environment, leading to reductions in HAIs rates [3].

In this respect, WHO proposed "My five moments for hand hygiene" which designates moments when HH is mandatory in healthcare assistance in an understandable and enlightening way. The five moments for HH are: 1) before touching a patient, 2) before clean/aseptic procedures, 3) after body fluid exposure risk, 4) after touching a patient and 5) after

touching patient surroundings [4]. Despite the proven efficacy of HH, compliance to this procedure around the world is far from ideal, in both developed and developing countries [5-7].

In addition to rates of HH based compliance based on opportunities, it is necessary to emphasize the relevance of verifying the quality of HH technique, which are related to correct steps and duration [8,9]. An observational study conducted at a teaching hospital in Switzerland evaluated 2,662 HH opportunities among healthcare professionals, as well as the fulfillment of the 6-step technique recommended by the World Health Organization (WHO), and found that the adherence rate was only 8.5% [10].

The HH technique recommended by WHO emphasizes six steps: (1) rub hand palm to palm; (2) right palm over left dorsum with interlaced fingers and vice versa; (3) palm to palm with fingers interlaced; (4) backs of fingers to opposing palms with fingers interlocked; (5) rotational rubbing of both thumbs; and (6) rotational rubbing of fingertips in the palm of the alternate hand [4].

However, studies indicated the possibility of streamlining WHO 6-step to three minimum steps and reported satisfactory results [11-13]. The three steps were: (1) cover all the surfaces of the hands; (2) rotational rubbing of fingertips in the palm of the alternate hand; and (3) rotational rubbing of both thumbs [11].

An experimental study showed that there was a significant logarithmic reduction in average bacterial counts when HH was performed according to the 3-step (median of 4.45, interquartile range 4.04 to 5.15) and that it even had a higher magnitude than that found for the WHO 6-step (median of 3.91, interquartile range 3.69 to 4.62, $p = 0.021$) [11]. These two groups represented intervention and control, respectively [11].

Another study where 2.923 HH opportunities were observed in more than 20 hospital wards found that adherence to five moments HH correct 3-step technique was considerably higher when comparing to WHO 6-step and the reduction factor of bacterial counts did not differ between the two techniques [12].

Regarding time, there is no consensus among researchers about the time required to perform the HH technique. An important study with healthcare professionals in a clinical setting regarding 3-step technique proved that 30 seconds alcohol-based hand rub (ABRH) rubbing time was considered effective [12]. However, a recent study conducted with 20 volunteers in Austria concluded that the 3-step HH hand rubbing for 15 seconds was not inferior to 30 seconds in reducing bacterial counts on hands [13].

The WHO guidelines indicate that HH can be executed with ABHR 20 to 30 seconds, or by washing the hands with water and soap for 40 to 60 seconds [4] and a recent systematic review concluded that there is not enough evidence to change WHO recommendations related to HH with ABHR application [14].

Given this scenario, it is important to emphasize that nursing professionals make up the greatest share of health teams, and have the closest contact with patients, because they execute care-related procedures that are associated with potential risk of dissemination of HAIs [15]. Therefore, the objective of the present study was to assess the effect of a correct 3-step hand hygiene technique on reducing of potentially pathogenic microorganisms on hands related to the WHO five moments for hand hygiene.

Methodology

Design and participants

This was a cross-sectional study, carried out by means of direct and nonparticipant observation. The study was carried out between February and June 2020, in the morning, afternoon, and night shifts in a clinical medicine unit and the adult Intensive Care Units (ICU) of a public teaching and research hospital in the Brazilian Center-West Region.

During the study period, there were eight beds in the adult ICU and 30 in the clinical medicine unit, and the study population was nursing workers, represented by nursing aides, nursing technicians, and nurses. The adult ICU nursing team was 25 nursing technicians and seven nurses, and the clinical medicine unit nursing team was nine nursing aides, 23 nursing technicians, and ten nurses.

The present study included professionals who carried out care-related activities oriented toward patients and who were not taking time off, or on vacation, maternity leave, work leave, or medical leave to treat a health problem, during the data collection period. Professionals who refused to participate in the study were excluded. Also excluded were those who had used steroids or topical or systemic antimicrobials during the last month previous to when they were approached about participating in the study, in order to guarantee that the microbiota of the hands of all the examined professionals was evaluated under similar conditions. Professionals whose hands showed impaired skin integrity, as a consequence of wounds, eczema, psoriasis, or other relevant skin injuries, were also excluded.

The Skin Conditions Evaluation Scale was used to objectively assess the skin of the participants. This instrument is in the Manual for Observers for the WHO multimodal HH improvement strategy, and it is applied to evaluate the presence of redness, squamosity, fissures, and scale [4].

Data collection

Data collection was carried out in four phases: direct observation, collection of samples from the hands of the professionals, application of a questionnaire with sociodemographic questions, and identification of the microorganisms found.

Prior to beginning of the observation period, to reduce bias, the specialist researcher responsible for the study, acted as a judge to verify the agreement between the researchers designated to perform observation/data collection. A specific training was conducted with realistic simulations in the clinical setting covering the five moments for HH: 1) before touching a patient, 2) before clean/aseptic procedures, 3) after body fluid exposure risk, 4) after touching a patient and 5) after touching patient surroundings.

The training was important to clarify any questions about each HH opportunity in order to identify the correct moment to perform sample collection and mainly to guarantee reliability and internal data validity. The training was based on the HH technical reference manual [4] to be used by healthcare workers, trainers and observers of HH practices and in this exercise a total of 53 HH opportunities were observed – all of them validated by the judge - involving ten nursing professionals, during the morning and afternoon shifts in the sectors where the study was to be performed.

In order to test interobserver agreement regarding the executed actions related to the five moments for HH, the judge compared both HH opportunities signaled in a validated WHO instrument [4] and the kappa coefficient was calculated [16,17]. The obtained value was 0.90, which is classified as nearly perfect agreement [18], which means that observers/researchers are qualified. It is important to highlight that the researcher responsible for HH observation acted as a nurse specialized in ICU and had ten years of professional experience in this field and, consequently, she had mastered the care routine in this setting, which contributed to a valid observation and collection of microbiological samples from the hands. The other qualified researcher supported microbiological collection and compared the number of each HH opportunities in order to assure quantitative balance.

Additionally, the researcher reported to the nurse in charge of the unit and the nursing team prior the beginning data collection. The nursing team was informed that direct observation of HH and microbiological collection of samples from the hands of the professionals would occur at random moments of the care practice. The researcher stayed in the sector for at least thirty minutes before initiating data collection, so the professionals could get used to her presence, which minimized the Hawthorne effect. Each professional was observed for about 30 minutes and microbiological collection was performed only once in each nursing professional.

During the observation process, the HH technique regarding time (seconds) and the correct 3-steps were verified and recorded, as well as whether the action was performed by ABHR or washing hands with water and soap or with chlorhexidine digluconate 2%. The time necessary to execute the technique was measured with a digital stopwatch, whose quality was attested by the Brazilian National Institute of Metrology, Quality and Technology.

For the evaluation of the 3- step hand hygiene technique, the procedure standardized as correct or satisfactory was judged by the 3-step proposed by Tschudin-Sutter *et al*. [11]: (1) cover all the surfaces of the hands; (2) rotational rubbing of fingertips in the palm of the alternate hand; and (3) rotational rubbing of both thumbs.

It is important to emphasize that professionals did not receive previous training on the 3-step HH technique. The researcher observed whether, during the HH procedure, the nursing professionals performed the 3-step of the reduced technique, since all these three steps are included in the Who 6-step complete technique.

Regarding HH execution time, the time considered correct was the recommended duration of 20 to 30 seconds for friction with ABHR and 40 to 60 seconds for sanitization with water and soap or chlorhexidine, recommended by WHO guidelines [4]. In this study, this time was standardized because there was no time consensus among researchers and a recent systematic review indicated that there is not enough evidence to change the WHO recommendations washing and rubbing time [14].

It is noteworthy that the ABHR used in the institution was a 70% ethanol solution, with neutral pH and odorless. The common liquid soap used also had a neutral pH. chlorhexidine digluconate was present in

the solution used at a concentration of 2%. The volume of the products used was not evaluated.

Collection of samples of the microbiota from the hands of the workers was carried out immediately after HH observation and filling WHO recommend form, at each of the five previously cited HH moments. The intention of collecting the microbiota of the hands of the nursing professionals at the moments in which HH was indicated during clinical practice was to verify HH effectiveness so that what microorganisms could be being transmitted to patients, the hospital environment, and the multiprofessional team. There were two people other than the observer and the microbiological researcher to help collect, identify, and readily store the samples.

Professionals were instructed to close their dominant hand and touch nothing until they got to the place where collection was carried out, which was next to the place where they carried out their activities. The dominant hand is used more often during daily practice and has higher chances of being associated with cross-transmission [19].

The researcher, wearing sterilized gloves, opened an autoclavable 20-L plastic bag, and the worker introduced their dominant hand in the bag, which contained 200 mL of brain heart infusion, and the hand was massaged for one minute against the bag wall. A solution to neutralize residual antiseptics was not used. This method was chosen because it guarantees collection of samples originating from the back of the hand, the palm, and the area between the fingers [20].

Microorganism isolation

The 0.1-mL aliquots from each sample of the brain heart infusion were seeded on Petri dishes containing culture media to select potentially pathogenic microorganisms: *Enterococci*-selective agar, cetrimide agar, eosin methylene blue agar, Mueller-Hinton agar supplemented with 4% NaCl and 6 µg/mL oxacillin, and potato dextrose agar with gentamicin. All the dishes were incubated at 36 ºC, and readings occurred 24 hours, 48 hours, and 72 hours after incubation. Colonies were identified according to their morpho staining characteristics (Gram staining) and by applying classic microbiology techniques. When the incubation period was over, the readings of the dishes were carried out according to the macroscopic morphology of the colonies.

Microorganism identification and susceptibility

Identification and antimicrobial susceptibility of the microorganisms isolated at the species level were carried out in the VITEK 2 Compact (bioMérieux®, France) system by following the manufacturer's instructions. The minimum inhibitory concentration for analysis of the susceptibility results was evaluated according to the 2018 Clinical & Laboratory Standards Institute guidelines [21].

Statistical analysis

Data were inserted in an electronic spreadsheet by means of double typing, and exploratory and inferential analyses were performed on R software [22].

The proportions of HH actions not carried out, correct and incorrect 3-step hand hygiene and microorganisms found on the hands of nursing professionals were compared with the variables indication, professional category and work sector by applying the chi-squared test [23]. The level of significance adopted in all analyses was 5%.

The variables analyzed for this study were: hand hygiene opportunities (the five moments for HH), professional category (nursing team members study participants: nurses, nursing technicians, nursing aides), work sector, action taken by the observed professional: correct 3-step HH technique (rubbing with ABHR, water and soap or chlorhexidine), HH not executed, HH executed incorrectly and presence of potentially pathogenic microorganisms.

Binomial analysis was carried out by using the Z-test to compare the proportions of hand hygiene actions not carried out and hand hygiene actions carried out incorrectly between the variables indication, professional category and work sector. The same test was applied to carry out a comparative analysis of the proportions of the microorganisms found on the hands of nursing professionals and the variables mentioned above. The level of significance adopted in all analyses was 5%.

Ethical aspects

The proposal for the present study was approved by a research ethics committee as per Presentation Certificate for Ethical Appreciation no. 75169317.0.0000.5541. The study was covered by all the legal principles for the protection of human subjects during the execution of studies.

Results

Of the 74 nursing professionals who worked in the examined sectors during the data collection period, five were excluded because they did not agree to participate in the study, two for showing skin injuries, two for being on leave for medical treatment, two for being on

vacation, and three for carrying out mostly administrative activities. Consequently, the final sample was 60 nursing professionals, among whom 11 (18.33%) were nurses, 45 (75%) were nursing technicians, and four (6.67%) were nursing aides, which corresponded to 81% of the population.

Thirty-five (58.3%) workers were women; 34 (56.7%) worked in the clinical medicine unit, and 26 (43.3%) worked in the ICU. Regarding age group, 31 (51.7%) professionals were between 31 and 40 years old, 17 (28.3%) were between 41 and 50 years old, seven (11.7%) were between 20 and 30 years old, and five (8.3%) were 51 years old or older.

Thirty-eight (63.3%) out of the 60 nursing professionals adhered to HH, but the cases in which the procedure was correctly applied considering the adequate time and the correct 3-step hand hygiene technique, the HH observed events dropped to only 13,3%. Compliance with the first step, "cover all the surfaces of the hands", was 100%. However, compliance to the second step, "rotational rubbing of fingertips in the palm of the alternate hand", occurred with 20 (52.6%) professionals, and the third step, "rotational rubbing of both thumbs", was executed by 12 (31.6%) professionals.

Regarding HH execution time, nine (27.3%) of the 33 professionals who washed their hands with water and soap or chlorhexidine carried out the procedure for adequate time, that is, more than 40 seconds. It is important to note that among those nine who performed the technique at the correct time, only six of them also performed the correct HH technique with soap and water. The minimum time spent to execute HH with water and soap or chlorhexidine was 10 seconds, and the maximum time was one minute and 9 seconds. The average value was around 31 seconds. Among the five workers who rubbed their hands with ABHR, only two (40%) executed the procedure for the correct duration and these two participants also performed the correct HH technique. The minimum time spent to perform HH with ABHR was 6 seconds, and the maximum time was 27 seconds. The average value was around 14 seconds. It's also important to highlight that of all the actions that were incorrectly carried out, 27 were performed with water and soap and only three of them were by means of ABHR ($p < 0.001$).

As shown in Table 1, it is emphasized that 66.7% of the nursing professionals who did not carry out HH failed to perform it at the moment "before clean/aseptic procedures." There were no statistical differences between HH indications and actions carried out. However, when the results obtained for the moments "before clean/aseptic procedures" and "before touching a patient" were compared with those for the moments "after body fluid exposure risk" and "after touching a patient", it was found that the "before" moments showed a higher proportion of actions not carried out ($p < 0.003$). In the group of actions carried out incorrectly, greater inadequacy in the technique was observed in the

Table 1. Frequency (f) and proportion (%) of nursing team members (n = 60) according to hand hygiene indication, professional category and work sector.

	Friction with ABHR Correctly (n = 2)		Water and soap or chlorhexidine Correctly (n = 6)		Not carried out (n = 22)		Carried out incorrectly (n = 30)		Total (n=60)	
	f	%	f	%	f	%	f	%	f	%
Hand hygiene Indication										
Before contact with patients	-	-	2	20.0	5	50.0	3	30.0	10	100.0
Before clean and aseptic procedures	1	8.3	-	-	8	66.7	3	25.0	12	100.0
After risk of exposure to body fluids	1	6.7	2	13.3	2	13.3	10	66.7	15	100.0
After contact with patients	-	-	1	8.3	2	16.7	9	75.0	12	100.0
After contact with areas next to patients	-	-	1	9.1	5	45.5	5	45.5	11	100.0
Professional category										
Nurse	-	-	2	18.2	3	27.3	6	54.5	11	100
Nursing technician	2	4.4	3	6.7	18	40.0	22	48.9	45	100
Nursing aide	-	-	1	25.0	1	25.0	2	50.0	4	100
Work sector										
Clinical medicine	1	2.9	3	8.8	13	38.3	17	50	34	100
Intensive care unit	1	3.8	3	11.5	9	34.6	13	50	26	100

ABHR: Alcohol-based hand rubs.

Table 2. Frequency (f) and proportion (%) of MR-CoNS that showed resistance to the tested antibiotics.

Antibiotics	*S. haemolyticus*		*S. epidermidis*		*S. warneri*		*S. saprophyticus*		*S. cohni spp urealyticus*	
	f	%	f	%	f	%	f	%	f	%
Benzylpenicillin	21	100	12	100	2	100	1	100	1	100
Oxacillin	21	100	12	100	2	100	1	100	1	100
Gentamicin	16	76.2	2	16.7	0	0	0	0	0	0
Levofloxacin	20	95.2	7	58.3	0	0	0	0	0	0
Erythromycin	21	100	10	83.3	1	50	1	100	1	100
Clindamycin	21	100	10	83.3	2	100	1	100	1	100
Rifampicin	14	66.7	4	33.3	2	100	0	0	0	0
Trimethoprim / sulfamethoxazole	12	57.1	4	33.3	0	0	1	100	0	0

"after" moments, and the difference was also statistically significant ($p < 0.007$).

Although comparison of HH actions indicated no statistically significant difference between professional categories and work sectors, it was noteworthy that 40% of the nursing technicians did not carry out HH (Table 1).

Among the 60 samples collected from the professionals' hands, 76.7% (46) showed positive growth for at least one potentially pathogenic microorganism. Sixty-five potentially pathogenic microorganisms were isolated and identified from these samples: 37 (56.9%) Coagulase Negative Staphylococci (CoNS) isolates, 17 (26.2%) Gram-negative bacilli (GNB), five (7.7%) *Enterococcus faecalis* isolates, four (6.2%) *Candida parapsilosis* isolates, one (1.5%) *Candida albicans* isolate, and one (1.5%) *Staphylococcus aureus* isolate.

In the group of Methicillin-resistant Coagulase Negative Staphylococci (MR-CoNS) isolates, 21 (51.4%) were identified as *Staphylococcus haemolyticus*, 12 (32.4%) as *Staphylococcus epidermidis,* two (5.4%) as *Staphylococcus warneri*, one (2.7%) as *Staphylococcus saprophyticus*, and one (2.7%) as *Staphylococcus cohni spp urealyticus*.

Regarding the GNB strains, six (35.3%) were *Acinetobacter ursingii*, two (11.8%) were *Enterobacter asburiae*, and two (11.8%) *Pantoea spp*. Additionally, *Pseudomonas aeruginosa*, *Acinetobacter baumanni complex*, *Acinetobacter iwoffi*, *Enterobacter aerogenes*, *Stenotrophomonas maltophilia*, *Bordetella hinzii*, and *Aeromonas sobria* contributed with one (6.25%) isolate each.

Regarding antimicrobial resistance, all CoNS were resistant to benzylpenicillin and methicillin and sensitive to linezolid, daptomycin, teicoplanin, vancomycin, tigecycline, nitrofurantoin, and streptomycin. In addition, all the CoNS isolates showed multidrug resistance (MDR), which is defined as resistance to at least three different classes of antimicrobial drugs [24] (Table 2).

Table 3 shows the resistance profile of the GNB found on the hands of nursing professionals in the present study.

Table 3. Resistance profile of the of Gram-negative bacilli[1] (n = 7) found on the hands of nursing professionals.

Antibiotics	*A. baumanni*	*A. iwoffi*	*A. sobria*	*E. aerogenes*	*E. asburiae*	*P. aeruginosa*
	(n = 1)	(n = 1)	(n = 1)	(n = 1)	(n = 2)	(n = 1)
Ampicillin	R	S	R	R	S	R
Cefuroxime	R	S	R	R	S	R
Cefuroxime Axetil	R	S	R	R	S	R
Cefoxitin	R	S	R	R	R	R
Ceftazidime	I	S	R	S	S	S
Ceftriaxone	I	S	R	S	S	R
Ampicillin / Sulbactam	S	S	R	R	S	R
Piperaciclin / tazobactam	R	S	R	S	S	S
Cefepime	S	S	R	S	S	S
Imipenem	S	S	R	I	S	S
Meropenem	S	S	R	S	S	S
Amikacin	S	S	R	S	S	S
Gentamicine	S	S	I	S	S	S
Ciprofloxacin	S	S	R	S	S	S
Tigecycline	S	S	S	S	S	R
Colistin	S	S	R	S	S	S

R: Resistant; S: Sensitive; I: Intermediate; [1]It was not possible to determine the resistance profile of *Pantoea spp*, *Stenotrophomonas maltophilia*, *A. ursingii*, and *Bordetella hinzii* isolates.

It was not possible to determine the resistance profile of *Pantoea spp*, *Stenotrophomonas maltophilia*, *A. ursingii*, and *Bordetella hinzii* isolates because the automatized system used in the present study does not perform susceptibility tests for these microorganisms.

The identified *Staphylococcus aureus* isolate was resistant to benzylpenicillin and sensitive to the other antibiotics tested. One out of the five *Enterococcus faecalis* found showed an MDR phenotypic profile, being resistant even to vancomycin, and the other four isolates were resistant to clindamycin and trimethoprim/sulfomethoxazole. Four isolates were sensitive to gentamicine, levofloxacin, erythromycin, teicoplanin, and vancomycin, and three were resistant to streptomycin. All the yeasts were sensitive to the tested antifungals: fluconazole, voriconazole, caspofungin, micafungin, amphotericin B, and flucytosine. It was not possible to determine the resistance profile of *Pantoea spp*, *Stenotrophomonas maltophilia*, *A. ursingii*, and *Bordetella hinzii* isolates because the automatized system used in the present study does not carry out susceptibility tests for these microorganisms.

Table 4 shows the distribution of different types of potentially pathogenic microorganisms according to professional category, work sector, HH moment and correct or incorrect HH technique.

The MR-CoNS isolates were found mostly in the nursing technician category and in the clinical medicine unit. Despite the highest prevalence on nursing technicians' hands, no significant difference was found between the value obtained for this category and that recorded for nurses (p = 0.07) and nursing aides (p = 0.2). Still in this context, the proportion of MR-CoNS found in the clinical medicine unit was significantly higher than that obtained for the ICU (p = 0.003). It is important to stress that 31 (91.2%) nursing professionals who worked in the clinical medicine unit provided care to four patients or more, whereas 24 (84.6%) nursing professionals who worked in the ICU assisted two patients, at most.

Regarding HH actions, the professionals who did not carry them out showed higher rates of contamination with MR-CoNS and *E. faecalis* (81.8% and 13.6%, respectively). It was noteworthy that 100% of the hands of nursing professionals who carried out the correct three-step hand hygiene technique did not show growth of potentially pathogenic microorganisms.

Table 4. Frequency (f) and proportion (%) of nursing team members (n = 60) according to hand hygiene indication, correct 3-steps technique, professional category and work sector.

Variables	MR-CoNS (n = 37)		*S. aureus* (n = 1)		*E. faecalis* (n = 5)		GNB (n = 17)		*Candida albicans* (n = 1)		*Candida parapsilosis* (n = 4)		Total (n = 60)	
	f	%	f	%	f	%	f	%	f	%	f	%	f	%
Hand hygiene Indication														
Before contact with patients	7	70.0	1	10.0	1	10	5	50.0	-	-	2	20.0	10	100.0
Before clean and aseptic procedures	8	66.7	-	-	2	16.7	1	8.3	-	-	1	8.3	12	100.0
After risk of exposure to body fluids	10	66.7	-	-	1	6.7	6	40.0	1	6.7	-	-	15	100.0
After contact with patients	6	50.0	-	-	1	8.3	3	25.0	-	-	1	8.3	12	100.0
After contact with areas next to patients	6	54.5	-	-	-	-	2	18.2	-	-	-	-	11	100.0
Correct 3-steps technique														
ABHR	-	-	-	-	-	-	-	-	-	-	-	-	2	100.0
Water and soap and chlorhexidine	-	-	-	-	-	-	-	-	-	-	-	-	6	100.0
Not carried out	18	81.8	-	-	3	13.6	6	27.3	1	4.5	2	9.1	22	100.0
Incorrect 3-steps technique	19	63.3	1	3.3	2	6.7	11	36.7	-	-	2	6.7	30	100.0
Professional category														
Nurse	4	36.4	-	-	1	9.1	2	18.2	-	-	-	-	11	100.0
Nursing technician	32	71.1	-	-	4	8.9	15	33.3	1	2.2	4	8.9	45	100.0
Nursing aide	1	25.0	1	25.0	-	-	-	-	-	-	-	-	4	100.0
Work sector														
Internal medicine	27	79.4	1	2.9	3	8.8	11	32.3	1	3.0	3	8.8	34	100.0
Intensive care unit	10	38.5	-	-	2	7.7	6	23.1	-	-	1	3.8	26	100.0

MR-CoNS: methicillin-resistant coagulase-negative *Staphylococcus*; GNB: Gram-negative bacilli; ABHR: Alcohol-based hand rubs.

Discussion

It is important to highlight that the findings from this study shows that HH observed events to its five moments were considerably low and the rate of professionals who carried out the HH procedure unsatisfactorily was three times higher than that of workers who executed the technique correctly, which impacts patient safety and poses an enormous economic burden to the health system. However, a systematic education based on three-step technique was not performed with healthcare professionals and another important and necessary element to consider is the accessibility of ABHR.

According to Stadler and Tschudin-Sutter [25], developments towards systematic education, better surveillance and streamlining of HH actions may improve compliance. The authors also stress that another important and necessary element to achieve compliance relies on ABHR accessibility. Even though it was not an objective of the present study, the actions performed with ABHR were considerably lower than actions with water and soap and it was noted by observers that ABHR were rarely available at the point of care, since there were beds with no ABHR supplies and some dispensers were not perfectly functioning or were not refilled.

Other studies that have assessed the quality of the HH procedure using the WHO six-step technique as a reference have shown even lower adherence rates, including 7.9%, 8.5%, and 9.5% [8,10,26], and authors conclude that ongoing and systematic education regarding HH techniques are mandatory.

In the present study we found that the most neglected steps in HH technique were " 2) rotational rubbing of fingertips in the palm of the alternate hand" and " 3-) rotational rubbing of both thumbs" which showed conformity rates of 52.6% and 31.6%, respectively. These rates were similar to those reported in previous studies with health professionals in hospital institutions in Korea and Qatar [8,27]. The study performed in Qatar found that conformity with the 2º step was 40.9%, decreasing to 30.3% for the 3º step, when executed with water and soap [27].

Regarding the time spent to execute HH, our study indicated that average time recorded was lower than that recommended by WHO - reference adopted in the present study. Other studies have found that health professionals execute HH procedures for insufficient durations, which makes the procedure's efficacy questionable [7,9,28]. If not all the surfaces of the skin are covered, or if the contact time of hygiene products is insufficient to guarantee adequate antimicrobial activity, the technical quality of HH and the delivery of safe care are compromised [11,27].

In the present study, it must be stressed that the "before" moments showed a higher proportion of actions not carried out ($p < 0.003$) when compared to after" moments. This result was similar to that reported in a survey with nurses in Iran, which found a significant difference ($p = 0.001$) between adherence rate to HH in pre-procedure indications (before touching a patient and before clean/aseptic procedure) and indications post-procedure (after contact with patient, after body fluid exposure risk), which were 3.40% and 21%, respectively. In line with the findings, authors concluded that HH is hardly perceived as a communal responsibility and healthcare works do not consider it as a "duty of care towards their patient" but as a "duty of care towards themselves" [25].

Previous studies have also obtained considerably numbers of health professionals' hands contaminated with microorganisms [29-32]. A study in Italy reported that 100 (5.41%) out of 1,848 samples collected from the hands of health professionals showed growth of some species of microorganisms [29]. In contrast, a study by Kalaiselvi and Padmavathi [30] found a rate of hands positive for potential pathogens higher than that of the present study, since microorganism growth was observed in 107 (86.3%) out of 124 health professionals' hand samples.

As reported in other studies [30,31] the microorganisms more often isolated from the hands of nursing professionals were CoNS. They are associated mostly with bloodstream infections and implanted medical devices [33]. The CoNS isolates examined in the present study showed an MDR phenotype, a worrying finding, given the high resistance of these microorganisms to antibiotics. This reinforces the characterization of CoNS isolates as pathogenic, which poses a challenge to infection treatment [34].

The examined CoNS isolates in our study showed a 100% resistance index to methicillin and all these strains were sensitive to vancomycin, which is the antibiotic commonly used to treat patients infected with MR-CoNS. However, vancomycin has potentially serious consequences, because it is administered intravenously only and can be nephrotoxic, an adverse effect that is associated with longer hospital stays, higher hospital costs, and a higher mortality rate [33,35]. Previous studies have found that CoNS isolates with a lower antibiotic resistance rate on the hands of health workers [29,30]. A study similar to the present one reported a methicilline resistance rate lower than that found in the present study, given that the resistance

calculated for 57 CoNS isolates collected from 124 health professionals' hand samples was 29.8% [30].

In the GNB group, *A. ursingii* isolates were the most frequent on the hands of nursing workers. Species of the *Acinetobacter spp*. genus were also the GNB more often isolated from the hands of health professionals in other studies worldwide [31,32]. Research in India showed that, among the 34 GNB found in health professionals' hand samples, 20 were *Acinetobacter spp*. isolates [32]. It is important to emphasize the presence of an *Aeromonas sobria* isolate that had a phenotypic MDR profile and can act as an opportunistic pathogen, causing intestinal infections and extraintestinal infections, such as skin and soft tissue infections, as well as bacteremia, regardless of the patient's immunological condition [36].

C. parapsilosis was the yeast species most found on the hands of our nursing professionals sample. A study carried out in Italy also verified that *C. parapsilosis* was the most common yeast on health professionals' hands, accounting for 57% of 77 isolates [37]. The same study, carried out over four years, reported a high bloodstream infection rate caused by *C. parapsilosis*. This species was present in 59.7% of the cases of this type of infection in three ICUs.

It should be stressed that microorganisms that are the main cause of HAIs worldwide, associated with longer hospital stays and high hospital mortality indexes, were found in the samples of the present study. Examples of these microorganisms include an *A. baumanni complex*, *P. aeruginosa*, vancomycin resistant Enterococci (VRE), and *S. aureus* isolates [38,39].

In the present study, the lower HH observed events in the clinical medicine unit impacted MR-CoNS rates. These findings may be related to the higher number of patients cared for per professional, which results in more HH moments and opportunities, however future studies must clarify associated factors. A study carried out with nursing professionals in China verified that the higher the work load, the lower the HH adherence rate ($p < 0.001$) [40].

Our findings observed that nursing technicians showed a higher rate of HH not executed, and a resulting higher rate of hands infected with MR-CoNS, corroborating results of preview study [30]. These results are worrying, given that the work of the nursing technician category involves more physical contact with patients, providing them with direct and constantly assistance and nursing care [40].

However, the results from this study found that 100% of the hands of nursing professionals who carried out the correct three-step hand hygiene technique did not show growth of potentially pathogenic microorganisms. The findings results are confirmed by previous studies that verified bactericidal efficacy in 3-step HH technique [11-13]. In this context, given that HH compliance is suboptimal in most studies and is considered the main strategy to prevent HAIs, streamlining 6-step HH technique may encourage higher overall adherence rates - because it is more simple and rational - and shows promising results related to quality and microbiological efficacy, which reinforce its potential to be considered the gold standard during healthcare assistance.

This research had limitations. The study represents a small sample, we were not free from Hawthorne effect and the study was performed in an ICU of a single institution. However, it was not possible to include other institutions because of budget constraints Brazil has been facing in research and scientific area, especially in recent years. We take advantage of this moment to reinforce our efforts to improve Brazilian scenario and we are more hopeful now.

Another possible limitation was not using a buffer solution to neutralize any residual antiseptic on the skin and help remove microorganisms. Additionally, the volume of products used when performing HH procedures was not measured. It is important to consider that the Hawthorne effect may be present in observational studies, which can lead to overestimation of conformity with HH procedures [41].

Larger and multicentric studies are needed, especially with controlled and randomized designs, in order to support the evidence indicated in this study and reinforce the clinical applicability of streamlining 6-step hand hygiene technique.

Conclusions

Adherence to the correct hand hygiene technique was considerably low, as there was poor adherence to rotational rubbing of fingertips and both thumbs. Also, time spent for the procedure was insufficient, with a consequent impairment of the technique effectiveness.

The results are worrying, because they indicated the presence of potentially pathogenic microorganisms, including MR-CoNS, GNB, *Enterococcus faecalis*, and *Candida parapsilosis* on the professional's hands, with a marked predominance of these microorganisms on the hands of nursing technicians, who are on the front line of nursing care in Brazil.

The present study showed that, when correctly executed in terms of both steps carried out (3-step technique) and antiseptic action time, hand hygiene can

be considered a preventive measure in pathogen dissemination, since hands that were adequately sanitized did not show growth of pathogenic microorganisms.

Streamlining hand hygiene technique consisting of three-step yielded encouraging results regarding technique efficacy and future randomized controlled trials are recommended in order to support the evidence indicated in this study and reinforce the clinical applicability in daily clinical practice.

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Authors' Contribuitions

Marília Duarte Valim, Emanuelle Righetto Corrêa, Alexandre Paulo Machado, Richarlisson Borges de Morais, Raoni Teixeira and Gerard Lacey each contributed equally to the design, the analysis and the interpretation of the data of the study, drafted the manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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