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ORIGINAL RESEARCH

Evaluating the Effectiveness of the WHO 6-Step Hand Hygiene Technique: Impact of Step Omission and Duration on Microbial Reduction

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Introduction: The World Health Organization has proposed practical recommendations for hand hygiene, which include a six-step technique for hand washing and hand rubbing with antiseptic agents. Hand hygiene has become one of the five key elements of the patient safety program. However, adherence to all six steps of hygienic handrub among medical staff is quite low. In our study, we evaluated the effectiveness of reducing microbial load on the skin of hands depending on the number of steps performed and the duration of handrub.

Methods: The preparation of the contamination fluid and the contamination of the volunteers' hand skin were carried out according to the European standard EN 1500. Subsequently handrub was performed using an alcohol-based antiseptic with various modifications of the six-step technique, either excluding certain elements or reducing the time spent on each step. After the handrub, swabs were performed, and the number of viable microorganisms in the obtained samples was determined.

Results: Adherence to all stages of the WHO-recommended 6-step hand hygiene technique provided effective decontamination of hand skin. Using all six steps for 30 seconds resulted in a log reduction of microorganisms up to 2.82 (mean value of colony-forming unit (CFU) = 2.024×10^3 (95% confidence interval (CI) $\pm0.545\times10^3$). Reducing the duration of antiseptic use from 30 to 15 seconds did not decrease the effectiveness of the antiseptic action.

Conclusion: Based on the conducted experimental studies, it can be concluded that following the WHO-recommended algorithm for hand hygiene significantly reduces the contamination of the skin surface with test cultures.

Keywords: hand hygiene, microbial load, hygienic handrub technique

Introduction

Healthcare-associated infections (HAIs) are among the most common adverse events occurring in the context of providing medical care to patients in healthcare facilities.[1] According to the World Health Organization (WHO) Global Report for 2021–2022 on infection prevention and control, during hospitalization, 7 out of 100 patients in high-income countries and 15 out of 100 patients in low- and middle-income countries acquire at least one healthcare-associated infection. On average, one in ten of these patients will die as a result of an HAIs.[2]

According to the European Centre for Diseases Control (ECDC), annually over 3.5 million cases of HAIs are reported in the European Union and European Economic Area (EU/EEA), resulting in more than 90,000 deaths. This constitutes a significant economic and social burden, surpassing the cumulative burden of other infections in the EU/EEA, including influenza and tuberculosis. Additionally, 71% of HAIs outbreak are caused by antibiotic-resistant bacterial strains.[3] A targeted study on the prevalence of healthcare-associated infections (HAIs) and the use of antimicrobial agents in European emergency hospitals in 2022–2023 revealed that the proportion of patients with at least one HAI in the EU/EEA sample was 7.1% (range for individual countries: 3.1–13.8%).[4]

The hands of healthcare workers are the most common means of transmitting pathogens from patient to patient and within medical environments. Therefore, hand hygiene is one of the primary measures to prevent the spread of

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antimicrobial resistance among microorganisms and reduce the likelihood of healthcare-associated infections in patients.[5] Compelling evidence demonstrates that implementing infection control measures, including hand hygiene programs, in healthcare facilities can achieve significant reductions in the incidence of HAIs, ranging from 35% to 70%.[6]

The normal microflora of the hands consists of two types of microorganisms: transient and resident flora.[7] Transient microflora of the skin of healthcare workers often includes microorganisms associated with HAIs. It is acquired by medical staff through contact with patients or environmental surfaces, leading to colonization of the superficial layers of the skin. Experimental studies have shown that after a single contact, approximately 4–16% of the total palm surface is affected, whereas after 12 contacts, this figure increases to around 40%.[8] There are various reports on the level of contamination of healthcare workers' hands. Some studies report contamination rates ranging from 10% to 78% among practicing physicians with strains of *S. aureus*, which is a predominant microorganism.[9] Studies found that in outpatient settings, healthcare workers acquired pathogens associated with healthcare-associated infections on their hands in 28.3% of medical encounters.[10]

When studying the efficiency coefficient of microorganism transfer during sequential surface contacts, it was shown that this coefficient was higher for bare hands (49%) compared to hands in gloves (30%). Microbial load typically reached a dynamic equilibrium after four and six contacts for gloved hands and bare hands, respectively. Individual differences between volunteers' hands had minimal impact compared to the use of gloves.[11] It was also shown that there is a direct correlation between bacterial load on healthcare workers' hands and the likelihood of pathogen transmission. Under specific conditions, there should be at least 1 $\log_{10}$ CFU present on healthcare workers' hands for transmission to occur during contacts.[12]

In 2005, the WHO launched the Global Patient Safety Challenge, and by 2009, they introduced the "5 Moments of Hand Hygiene" to reduce the spread of healthcare-associated infections within healthcare settings.[13] WHO recommends implementing the 6-step hand hygiene technique (Figure 1) in healthcare facilities, which was proposed by Professor Graham Ayliffe as early as 1978 to standardize this procedure.[14,15] The 6-step technique is now globally accepted as the gold standard for handwashing with soap and water, as well as for hand-processing with antiseptic agents in clinical practice.[13] However, compliance with this technique in healthcare facilities across different countries remains low.[16,17] One possible way to improve compliance with this technique is to provide healthcare workers with evidence of how effective the recommended 6-step technique is for decontaminating the skin of hands and covering all their surfaces.[18] Despite global use of WHO recommended approach for hand hygiene, there is limited data published on reduction of transient microflora depending on duration of handrub and implementation of various steps for hand hygiene.

The presented study investigated the effectiveness of the World Health Organization's 6-step hand hygiene technique for reducing microbial load. The study also assessed the impact of deviations (such as omitting certain steps or reducing the duration of their execution) during handrub on its effectiveness.

Materials and Methods

Antiseptic Agents

WHO recommends alcohol-based antiseptics for hand hygiene based on the following factors: evidence-based, rapid onset and broad-spectrum microbicidal activity with a minimal risk of generating resistance to antimicrobial agents; suitability for use in resource-limited settings or remote areas; ability to improve adherence to hand hygiene requirements; economic benefits; and minimizing risks of adverse effects.[13] Based on the information provided, the antiseptic for the study was prepared in the laboratory Department of Microbiology and Parasitology with Basics of Immunology using recommendations outlined in the "Guide to local production: WHO-recommended handrub formulations". The formulation recipe is as follows: ethanol 96% – 833.3 mL; hydrogen peroxide 3% – 41.7 mL; glycerol 98% – 14.5 mL; water – 110.5 mL.[19] The use of alcohol-based hand sanitizers has proven its effectiveness in practice. Multivariate survival analysis showed a significant reduction in hospital-acquired infections/systemic inflammatory response syndrome cases with the use of alcohol-based hand sanitizers in pediatric and surgical departments.[20]

The effectiveness of the prepared antiseptic was evaluated according to the requirements of standard EN 13727:2012 + A2:2015, IDT.[21] This standard pertains to chemical disinfectants and antiseptics, specifically for evaluating bactericidal activity in medical settings using a quantitative suspension test method. The antiseptic was considered effective if it achieved a log reduction of test culture of at least 5.0.

Figure 1 World Health Organization's 6-step technique for hand hygiene with alcohol-based antiseptics.

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The Test Culture

The study used the test microorganism *E. coli* K-12. This strain originates from normal flora and is recognized as non-pathogenic. According to the NCIMB catalog, strain NCIMB 10083 is classified as a microorganism belonging to risk group 1. Storage and preparation of the initial culture of the test microorganism were conducted in accordance with standard EN 13727.[21]

Research on the Effectiveness of Different Hand Hygiene Techniques Using Alcohol-Based Antiseptic to Reduce Their Microbial Contamination

The 6-step hand hygiene method was initially developed for standardizing the testing of antimicrobial (antiseptic) agents used for hygienic hand processing, rather than for practical application in clinical settings.[14] Accordingly, research on the effectiveness of various hand hygiene techniques was conducted according to the requirements of the European standard EN 1500:2013 Chemical disinfectants and antiseptics — Hygienic handrub - Test method and requirements (phase 2/step 2), with some modifications.

To determine the effectiveness of the 6-step hand hygiene method and assess the impact of skipping specific steps on the reduction of microorganisms, the following experimental model was developed:

I. Enrollment of study participants (10 volunteers were enrolled).
II. Training of study participants in hand hygiene technique according to WHO recommendations.
III. Artificial contamination of volunteers' hands with *E. coli* K-12 using an contamination fluid. Preparation of working culture of test microorganisms, contamination fluid, and hand contamination were conducted according to EN 1500.
IV. Rubbing of skin with alcohol-based antiseptic. The handrub procedure involved combinations of different steps of the 6-step WHO hand hygiene method using 3 mL of handrub for each procedure (Figure 1 and Table 1). Each technique was tested in 10 repetitions per volunteer to accurately determine the remaining bacterial counts after treatment.
V. Sampling of the test organisms from all hand surfaces after treatment. Immediately after completing handrub with one of the combinations, samples were taken from the skin surface using cotton swabs. After sampling, the

Table 1 Combinations of Different Stages of Hand Handrub Techniques

Steps Performed According to WHO Recommendations	Technique
2 Step; 30 sec	No. 1
2–3 Step; 30 sec	No. 2
2–4 Step; 30 sec	No. 3
2–5 Step; 30 sec	No. 4
2–6 Step; 30 sec	No. 5
2–7 Step; 30 sec	No. 6
2 Step; 15 sec	No. 7
2–3 Step; 15 sec	No. 8
2–4 Step; 15 sec	No. 9
2–5 Step; 15 sec	No. 10
2–6 Step; 15 sec	No. 11
2–7 Step; 15 sec	No. 12
Control wash	–

swabs were placed in a vial with TSB medium (using the dilution procedure to stop the antiseptic action by reducing its concentration). The sampling scheme is shown in Figure 2. A series of ten-fold dilutions was prepared from the obtained sample, after which each dilution was plated on TSA medium. The interval between sampling and plating on the dishes did not exceed 30 minutes.

VI. Determination of the number of test microorganisms in the rinses. All concentration values of microorganisms are converted to decimal logarithm (lg). For each combination of different stages, the results were averaged. Then, for each combination, the decimal logarithm reduction was calculated separately using the formula:

$$\lg R = \lg \text{control} - \lg \text{combination of stages}$$

Results

During the first stage of the study, the antiseptic solution for handrub according to the WHO recommendations,[19] was prepared and tested for its antibacterial properties against the test strain *E. coli* K-12 according to the requirements of EN 13727:2012. The experimental results confirmed a high bactericidal activity of the prepared antiseptic agent, with a log reduction of 5.31.

After confirming the bactericidal activity of the prepared antiseptic, the second stage of the study focused on determining the effectiveness of various hand rubbing techniques in reducing microbial contamination of hand skin. The experimental data obtained are presented in Tables 2 and 3, as well as in Figures 3 and 4.

The most significant reduction in test culture concentration in rinses compared to the previous stage was observed with techniques No. 8 (log reduction = 1.725; mean value of CFU = 2.517×10^4) and No. 2 (log reduction = 1.807; mean

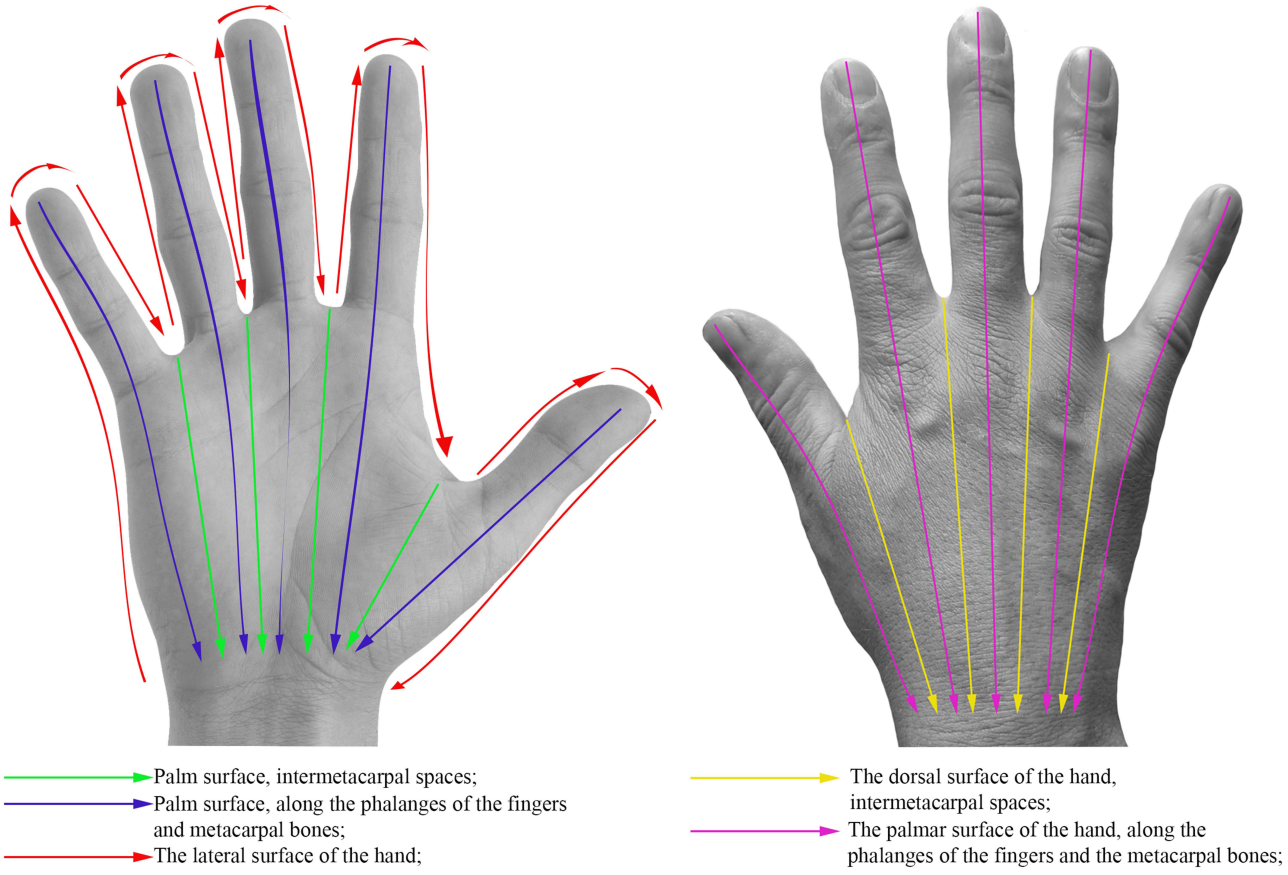

Figure 2 Scheme of sampling from the surface of the skin of the hands.

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Table 2 The Effectiveness of Hand Hygiene Using the Antiseptic for 30 seconds

Technique/ No.	2 Step; 30 sec	2–3 Step; 30 sec	2–4 Step; 30 sec	2–5 Step; 30 sec	2–6 Step; 30 sec	2–7 Step; 30 sec	Control
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	
Mean value of CFU	1.13×10^{5}	2.084×10^{4}	1.645×10^{4}	9.05×10^{3}	2.34×10^{3}	2.024×10^{3}	1.337×10^{6}
Standard deviation of mean value	0.889×10^{5}	2.592×10^{4}	1.255×10^{4}	9.0×10^{3}	0.957×10^{3}	0.879×10^{3}	0.637×10^{6}
Confidence Interval	$1.13\pm0.552\times10^{5}$	$2.084\pm1.607\times10^{4}$	$1.645\pm0.778\times10^{4}$	$9.05\pm5,58\times10^{3}$	$2.34\pm0.595\times10^{3}$	$2.024\pm0.545\times10^{3}$	$1.337\pm0.395\times10^{6}$
Log_{10}	5.053	4.319	4.216	3.957	3.369	3.306	6.126
Log reduction	1.073	1.807	1.91	2.169	2.757	2.82	–

Table 3 The Effectiveness of Hand Hygiene Using the Antiseptic for 15 seconds

Technique/ No.	2 Step; 15 sec	2–3 Step; 15 sec	2–4 Step; 15 sec	2–5 Step; 15 sec	2–6 Step; 15 sec	2–7 Step; 15 sec	Control
	No. 7	No. 8	No. 9	No. 10	No. 11	No. 12	
Mean value of CFU	6.34×10^{4}	2.517×10^{4}	1.918×10^{4}	1.877×10^{4}	6.99×10^{3}	2.039×10^{3}	1.337×10^{6}
Standard deviation of mean value	6.15×10^{4}	4.21×10^{4}	1.503×10^{4}	1.307×10^{4}	7.56×10^{3}	1.435×10^{3}	0.637×10^{6}
Confidence Interval	$6.34\pm3.81\times10^{4}$	$2.517\pm2.609\times10^{4}$	$1.918\pm0.932\times10^{4}$	$1.877\pm0.810\times10^{4}$	$6.99\pm0.469\times10^{3}$	$2.039\pm0.889\times10^{3}$	$1.337\pm0.395\times10^{6}$
Log_{10}	4.802	4.401	4.283	4.273	3.844	3.309	6.126
Log reduction	1.324	1.725	1.843	1.853	2.282	2.817	–

value of CFU = 2.084×10^{4}). This is attributed to the fact that these techniques involve treating the palmar and dorsal surfaces of the hand, as well as partially the interdigital spaces, which constitute over 80% of the total hand surface area (Tables 2 and 3).

The greatest reduction in skin contamination compared to the control, and the most consistent results, were observed with technique No. 6, which included all six WHO-recommended steps of hand skin treatment over a duration of

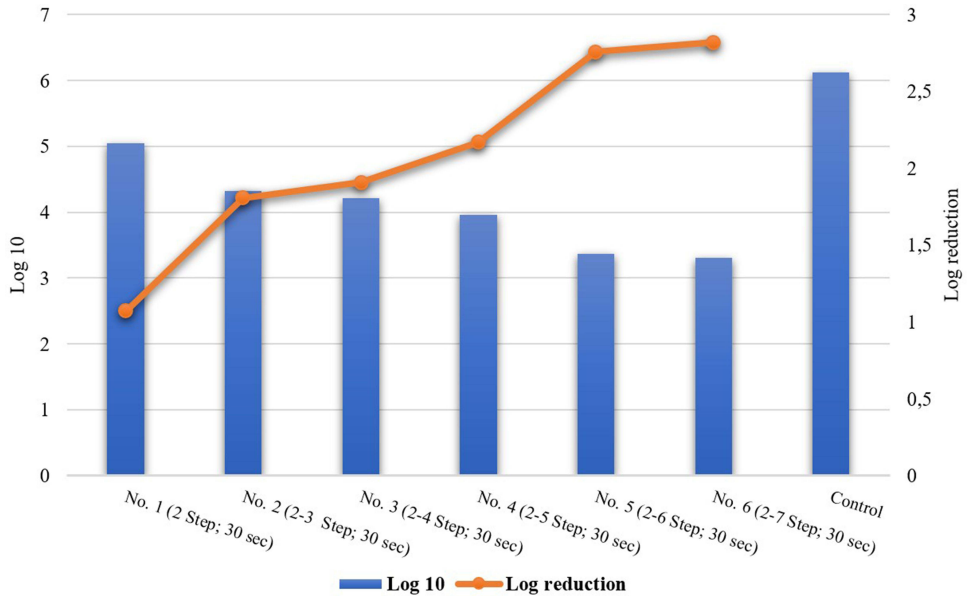

Figure 3 Concentration of test culture in rinses depending on the number of handrub steps (30 sec).

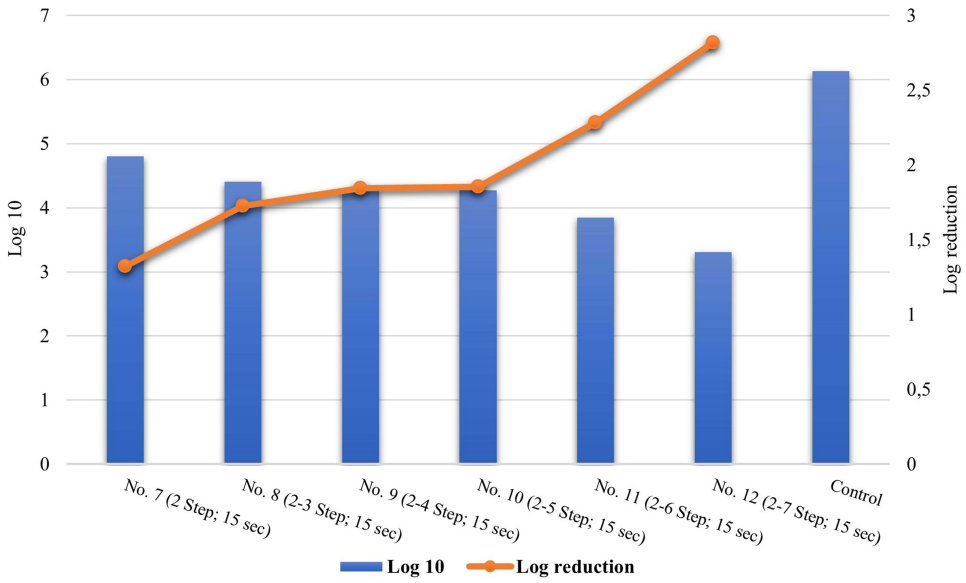

Figure 4 Concentration of test culture in rinses depending on the number of handrub steps (15 sec).

30 seconds. Conversely, the least reduction in microorganism count in rinses was observed with technique No. 1, which involved treating only the palmar surface of the hand for 30 seconds (Table 2).

Overall, there was a gradual decrease in the number of microorganisms in samples as the number of stages used for hand-processing with the antiseptic increased. Using only step No. 1 resulted in a log reduction of 1.073 (mean value of CFU =1.13×10^5 (95% CI $\pm0.552\times10^5$)), whereas employing all six steps for 30 seconds increased the log reduction to 2.82 (mean value of CFU = 2.024×10^3 (95% CI $\pm0.545\times10^3$)). In general, adherence to all six steps of the WHO 6-step hand hygiene method ensured effective decontamination of the skin (Figure 5).

Based on the above, our next goal was to investigate the impact of reducing the hand antiseptic treatment time from 30 to 15 seconds on the effectiveness of skin decontamination.

The experimental studies have shown that reducing the duration of antiseptic use from 30 to 15 seconds did not diminish the effectiveness of hand rubbing (Figures 3 and 4). Overall, the trends in reducing microbial contamination of the skin with *E. coli* K-12 using different techniques for 15 and 30 seconds were similar. Adhering to all six steps of the WHO 6-step hand hygiene method for 15 seconds resulted in a log reduction of 2.817 (mean value of CFU = 2.039×10^3 (95% CI $\pm0.889\times10^3$)), whereas using the antiseptic for 30 seconds resulted in a log reduction of 2.82 (mean value of CFU = 2.024×10^3 (95% CI $\pm0.545 \times 10^3$)).

Technique No. 7
(dilution of rinse 10^{-2})

Technique No. 12
(dilution of rinse 10^{-2})

Control
(dilution of rinse 10^{-2})

Figure 5 Microbial load in hand rinses using different handrub techniques.

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Discussion

Studies conducted in many healthcare facilities indicate that adherence to hand hygiene rules among medical workers remains low, ranging from 5% to 89%, with an average compliance rate of approximately 38%.[17] For example, in a study on the implementation of hand hygiene techniques in 15 different departments of healthcare facilities in Switzerland, it was shown that compliance with hand hygiene recommendations was 93.2% of all possible situations. No differences among professions were found. However, the completion of all six steps of the hand hygiene technique was only 8.5%, with most healthcare workers adhering to steps 2 and 3 only.[16] Monitoring the use of hand hygiene techniques showed that, overall, only the first three steps of the method were performed properly by the medical staff. After that, the compliance rate dropped to 79.9% at step 4, and to 14.9% and 36.6% at steps 5 and 6, respectively.[22] At the same time, it was shown that there is a significant decrease in the frequency of isolating antibiotic-resistant bacteria from clinical cultures in healthcare facilities where the greatest increase in hand hygiene compliance was observed.[23]

If certain areas of the skin are missed during hand treatment, it can limit the effectiveness of the entire procedure because live microorganisms may persist on these areas, potentially causing healthcare-associated infections.[24] Additionally, only the final steps of the WHO 6-step technique specifically address the fingertips (Figure 1), despite them being commonly colonized during medical care. Therefore, the primary aim of the study was to assess the impact of omitting specific steps of hand-processing on reducing skin microbial contamination. We aimed to evaluate whether skipping certain steps of the WHO 6-step technique would result in less reduction of bacteria on hands.

For alcohol-based hand hygiene products used according to WHO guidelines, the recommended duration of application is 30 seconds. However, this is often longer than the actual time applied in clinical practice. Several studies have shown that reducing the application time of antiseptics on hands can increase compliance among personnel while maintaining a similar microbiological effect. For instance, in a study on the microbiological safety of reducing the antiseptic application time to 15 seconds in a neonatal intensive care unit, it was found that reducing the application time to 15 seconds had a comparable microbiological effect to the 30-second hand processing. All tested hand hygiene products demonstrated similar effectiveness within 15 seconds compared to the standard 60% propan-2-ol concentration used for 30 seconds.[25] In another study, the effectiveness of alcohol-based hand sanitizers was tested for 15 and 30 seconds by measuring microbial load on fingertips and hand hygiene compliance among nurses in a gynecological department. The study found no difference in bacterial load on fingertips between the 15-second and 30-second application times. Participants who used the 15-second hand-processing were more likely to adhere to hand hygiene compared to those using the 30-second application. The frequency of handrub increased from 54.7% to 69.5% in the 15-second study.[26]

A large-scale assessments of hand hygiene quality showed frequent failure to adequately clean dorsal and palmar parts of the hand even after training by providers.[27] Our study shows that these elements of hand rubbing are critical for reduction of bacterial load.

Our study also has several limitations. An important limitation is that it evaluated the effectiveness of handrub techniques used by volunteers under experimental conditions, thus our results may only be partially extrapolated to clinical settings where hand hygiene is used to eliminate various transient microflora acquired during patient care. We tested only 1 strain of microorganism (*E. coli* K-12) and 1 type of antiseptic, which limits the generalizability of our findings to all possible types of antiseptic agents and various pathogenic and opportunistic microorganisms capable of causing HCAIs. Additionally, the study did not account for the surface area of the volunteers' hands participating in the study, which could affect the amount of microorganisms adhering to the skin surface upon contact with the disinfectant solution and subsequently recovered in the rinses.

Further research in this direction could aim to determine the microbiological effectiveness of the 6-step hand hygiene procedure in hospital settings, considering the microorganisms acquired by healthcare workers during patient care.

Non-adherence to the stepwise hand hygiene procedure, on the other hand, only leads to partial elimination of contamination and consequently increases bacterial load on the skin surface. This could be a significant contributing factor in healthcare settings, contributing to the spread of nosocomial infections and increasing cases of HAIs.

The experimental study also provides evidence supporting the rationale for reducing the hand hygiene procedure time to 15 seconds, which could be implemented in practice to increase compliance with the 6-step hand hygiene procedure among healthcare personnel in hospital settings.

Conclusion

Based on the experimental data obtained, it can be concluded that reducing the application time of the antiseptic to 15 seconds during hand hygiene should be considered as a critical component of a successful multimodal intervention strategy to improve hand hygiene compliance in clinical practice. Based on the conducted experimental studies, it can be concluded that following the WHO-recommended algorithm for hand hygiene significantly reduces the contamination of the skin surface with test cultures.

Ethics Approval

This study was conducted as part of a PhD research project with the approval of the Commission on Bioethical Expertise and Research Ethics of the Bogomolets National Medical University, in accordance with the principles outlined in the Declaration of Helsinki. Informed consent was obtained from all participants involved in the study.

Disclosure

The authors report no conflicts of interest in this work.

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