Chlorhexidine Handwash 2% Demonstrates Superior Effectiveness in Reducing Hand Microorganisms

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ABSTRACT
Healthcare-associated infections remain a major global challenge, with hand hygiene recognized as the most effective preventive measure. Chlorhexidine-based formulations are widely used, yet comparative evidence between different concentrations and modalities, particularly chlorhexidine gluconate 0.5% with 70% ethyl alcohol handrub and chlorhexidine 2% handwash remains limited. Understanding their relative effectiveness in reducing microbial load on the hands of healthcare workers is essential for optimizing infection control practices. This study aimed to compare the effectiveness of chlorhexidine gluconate 0.5% with 70% ethyl alcohol handrub and chlorhexidine 2% handwash in reducing bacterial colony counts on the hands of healthcare personnel. An experimental Pre–Post Test Group Design was conducted involving 20 respondents at the Microbiology Laboratory of Sultan Agung Islamic Hospital, Semarang. The handrub procedure was performed on day one and the handwash procedure on day two. Respondents included nurses, laboratory analysts, and office staff who met inclusion criteria and provided informed consent. Hand samples were collected using the glove-juice method, cultured on nutrient agar, and incubated for 24 hours. Percentage reduction in colony counts was calculated for each respondent. Data were analyzed using the Independent Sample t-Test. Female respondents predominated (85%). The mean percentage reduction in bacterial colonies was 72.79% for the handrub group and 82.57% for the handwash group. Statistical analysis demonstrated a significant difference between the two modalities ($p = 0.000$), indicating superior effectiveness of chlorhexidine 2% handwash. The microorganisms identified in pre- and post-intervention cultures included *Bacillus* spp., coagulase-negative *Staphylococcus* (CoNS), and *Stenotrophomonas maltophilia*. In conclusion, chlorhexidine 2% handwash was significantly more effective than chlorhexidine gluconate 0.5% with 70% ethyl alcohol handrub in reducing bacterial colony counts on the hands of healthcare workers. These findings support the preferential use of chlorhexidine 2% handwash in settings requiring maximal microbial reduction.
Keywords: hand hygiene; chlorhexidine gluconate; ethyl alcohol; infection control

INTRODUCTION
Healthcare-associated infections (HAIs) are infections that emerge in patients receiving medical care within healthcare facilities and are commonly transmitted through cross-contamination during routine clinical activities such as lifting patients, measuring pulse rate, blood pressure, or body temperature [1,2]. The World Health Organization (WHO) reports that the global prevalence of HAIs ranges between 7% and 22%, with Southeast Asian countries, including Indonesia showing disproportionately high rates. Indonesia has been identified as having one of the highest prevalence levels, reaching 30.4% [3]. These data underscore the urgent need for effective preventive strategies, among which hand hygiene remains the most fundamental and evidence-based intervention [4].

Despite its importance, comparative evidence on the effectiveness of handrub containing chlorhexidine gluconate 0.5% with 70% ethyl alcohol versus handwash containing chlorhexidine 2% in reducing microbial colony counts on hands remains limited. Existing studies have predominantly focused on chlorhexidine 4% formulations, particularly within surgical settings. However, the use of 4% chlorhexidine may increase the risk of dermatitis with frequent application and is associated with higher production costs for antiseptic solutions [5,6]. These limitations highlight the need to explore alternative concentrations that may offer comparable or superior antimicrobial efficacy with fewer adverse effects.

Alcohol-based handrubs exert antimicrobial activity by disrupting lipid membranes and denaturing proteins, ultimately compromising microbial membrane integrity and inhibiting metabolic processes [7]. Similarly, chlorhexidine-based handwash solutions act by disrupting the cytoplasmic membrane structure through interactions with phosphate-containing components of the bacterial cell wall [8]. Ineffective hand hygiene practices among healthcare workers are estimated to contribute to approximately 40% of nosocomial infections, emphasizing the critical role of proper technique and product selection [9]. The effectiveness of hand hygiene products depends on several factors, including the amount applied, duration of exposure, frequency of use, and the susceptibility of microorganisms present on the hands to the active ingredients [10].

Previous studies have shown that handwash with chlorhexidine 4% and handrub with 70% isopropyl alcohol do not differ significantly in reducing microbial colony counts, suggesting that both modalities may be effective under certain conditions. Handwashing is generally recommended when hands are visibly soiled, whereas handrubbing offers advantages in terms of time efficiency [11]. In contrast, Girou et al. reported that an alcohol-based handrub formulation (45% 2-propanol, 30% 1-propanol, and 0.2% mecetronium ethyl sulphate) demonstrated superior effectiveness compared to chlorhexidine gluconate 4% handwash [12]. These findings indicate that alcohol-based formulations may outperform chlorhexidine-based washes under specific circumstances.

Hand hygiene is widely recognized as the most effective measure for controlling nosocomial infections [13]. This evidence forms the rationale for conducting the present study, which aims to determine the difference in effectiveness between chlorhexidine gluconate 0.5% with 70% ethyl alcohol handrub and chlorhexidine 2% handwash in reducing the percentage of bacterial colony counts on the hands.

METHODS
The study was conducted in the Microbiology Laboratory of Sultan Agung Islamic Hospital, Semarang, during March 2024. The controlled laboratory setting ensured standardized environmental conditions for sample collection, intervention administration, and microbial culture procedures. This research employed an experimental design using a Pre–Post Test Group Design, aimed at determining the comparative effectiveness of two hand hygiene modalities: handrub containing chlorhexidine gluconate 0.5% with 70% ethyl alcohol and handwash containing chlorhexidine 2%. The design allowed measurement of microbial colony reduction before and after the intervention within each group, thereby enabling evaluation of treatment effects with minimal confounding.

Study subjects were recruited through consecutive sampling, involving healthcare personnel at Sultan Agung Islamic Hospital who were capable of performing hand hygiene according to the WHO six-step technique and who provided informed consent to participate. A total of 20 respondents were enrolled, consisting of nurses, laboratory analysts, and administrative staff. Participants were screened to ensure the absence of hand injuries, long fingernails, or allergies to alcohol or chlorhexidine, thereby minimizing factors that could interfere with the intervention or compromise safety.

The independent variable consisted of two hand hygiene treatments. The handrub formulation contained chlorhexidine gluconate 0.5% combined with 70% ethyl alcohol, prepared according to standard antiseptic specifications and applied following WHO handrub technique. The handwash formulation contained chlorhexidine 2%, prepared as a liquid antiseptic soap and administered using WHO handwash steps under running water. Both products were provided in sterile containers, and respondents were instructed to apply each treatment according to standardized duration and technique to ensure uniform exposure.

The dependent variable was the percentage reduction in bacterial colony counts on the hands following treatment. Microbial sampling was performed using the glove-juice method, in which each respondent inserted a hand into a sterile glove containing 50 mL of 0.9% NaCl solution. A tourniquet was applied at the wrist, and the hand was massaged for one minute to dislodge resident microorganisms. After releasing the tourniquet, 1 mL of the rinse solution was aspirated using a sterile syringe and streaked onto nutrient agar plates. The plates were incubated for 24 hours, after which colony counts were recorded. Sampling was conducted twice for each respondent, before and after the assigned hand hygiene intervention allowing calculation of the percentage reduction in colony numbers.

Data analysis was performed using SPSS version 26.0 for Windows. The percentage reduction in bacterial colonies between pre- and post-intervention measurements was calculated for each participant. Differences in effectiveness between the alcohol-based handrub group and the chlorhexidine 2% handwash group were analyzed using the Independent Sample t-Test, providing statistical comparison of mean reductions across the two treatment modalities.

Ethical principles were upheld throughout the study, including voluntary participation, informed consent, confidentiality of respondent data, and adherence to biosafety procedures during sample collection and laboratory processing. All procedures complied with institutional and national guidelines for research involving human participants.

RESULTS

Basen on Table 1, a total of 20 respondents participated in this study. Female respondents constituted the majority, with 17 participants compared to 3 male participants. The study was conducted over two consecutive days, with the handrub procedure administered on the first day and the handwash procedure on the second day. The interventions consisted of handrub containing chlorhexidine gluconate 0.5% with 70% ethyl alcohol and handwash containing chlorhexidine 2%.

Analysis of the percentage reduction in bacterial colony counts following the use of handrub chlorhexidine gluconate 0.5% with 70% ethyl alcohol and handwash chlorhexidine 2% demonstrated a statistically significant difference, with a p-value of 0.000 (Table 2). These findings indicate that the handwash method exhibited superior effectiveness compared to the handrub method. The microorganisms identified in both pre- and post-intervention cultures included *Bacillus* spp., coagulase-negative *Staphylococcus* (CoNS), and *Stenotrophomonas maltophilia* (Table 3).

Table 1. Distribution of demographic characteristics of respondents

Demographic variable	Frequency	Percentage
Sex		
Male	3	15
Female	17	85
Age		
26–35 years	3	15
36–45 years	11	55
46–56 years	6	30
Occupation		
Laboratory analyst	6	30
Nurse	9	45
Office staff	5	25

Table 2. Reduction in bacterial colony counts using handrub chlorhexidine gluconate 0.5% with 70% ethyl alcohol and handwash chlorhexidine 2%

Respondent	Handrub chlorhexidine gluconate 0.5% + 70% ethyl alcohol			Respondent	Handwash Chlorhexidine 2%		
	Pre	Post	Percentage reduction		Pre	Post	Percentage reduction
1	542	115	78.8	1	10	2	80
2	61	15	75.4	2	69	15	78.26
3	10	4	73.3	3	13	3	76.92
4	32	10	68.7	4	74	0	100
5	76	22	71	5	82	18	78.04
6	40	10	75	6	9	1	88.88
7	19	4	78.9	7	7	1	85.71
8	10	3	70	8	20	4	80
9	72	20	72.2	9	56	10	82.14
10	102	29	71.5	10	103	25	75.72
11	63	17	73	11	53	10	80.39
12	26	7	73.1	12	1	0	100
13	107	30	71.9	13	67	13	80.59
14	7	2	71.4	14	14	2	85.71
15	6	2	66.7	15	10	3	70
16	102	24	76.5	16	8	1	87.5
17	302	80	73.5	17	55	12	78.18
18	50	14	72	18	47	12	74.46
19	353	101	71.4	19	23	5	78.26
20	28	8	71.4	20	21	2	90.57
Mean	72.79			Mean	82.57		

Table 3. Culture Results Before and After Hand Hygiene

Respondent	Handrub chlorhexidine gluconate 0.5% + 70% ethyl alcohol		Respondent	Handwash chlorhexidine 2%	
	Pre	Post		Pre	Post
1	*Bacillus* sp., CoNS	CoNS	1	*Bacillus* sp., CoNS	CoNS
2	*Bacillus* sp., CoNS	CoNS	2	*Bacillus* sp., CoNS	CoNS
3	*Bacillus* sp., CoNS	CoNS	3	*Bacillus* sp., CoNS	CoNS
4	*Bacillus* sp., CoNS	CoNS	4	*Stenotrophomonas maltophilia*	–
5	*Bacillus* sp., CoNS	CoNS	5	CoNS	CoNS
6	CoNS	CoNS	6	CoNS	CoNS
7	CoNS	CoNS	7	*Bacillus* sp., CoNS	CoNS
8	CoNS	CoNS	8	CoNS	CoNS
9	CoNS	CoNS	9	CoNS	CoNS
10	CoNS	CoNS	10	CoNS	CoNS
11	*Bacillus* sp., CoNS	*Bacillus* sp., CoNS	11	CoNS	CoNS
12	CoNS	CoNS	12	*Bacillus* sp.	–
13	CoNS	CoNS	13	CoNS	CoNS
14	*Bacillus* sp.	*Bacillus* sp.	14	*Bacillus* sp., CoNS	CoNS
15	CoNS	CoNS	15	CoNS	CoNS
16	CoNS	CoNS	16	CoNS	CoNS
17	*Bacillus* sp., CoNS	*Bacillus* sp., CoNS	17	*Bacillus* sp., CoNS	CoNS
18	*Bacillus* sp., CoNS	*Bacillus* sp., CoNS	18	*Bacillus* sp., CoNS	CoNS
19	CoNS	CoNS	19	CoNS	CoNS
20	*Bacillus* sp., CoNS	*Bacillus* sp., CoNS	20	CoNS	CoNS

DISCUSSION

This study employed handrub containing chlorhexidine gluconate 0.5% with 70% ethyl alcohol and handwash containing chlorhexidine 2%, representing a methodological distinction from previous investigations that predominantly compared alcohol-based handrubs with chlorhexidine 4% handwash. Earlier studies reported no significant difference between chlorhexidine 4% handwash and 70% isopropyl alcohol handrub in reducing bacterial colony counts [11]. However, the use of 4% chlorhexidine is associated with a higher prevalence of skin sensitization and irritation, in addition to increased production costs for antiseptic formulations [5,14]. These limitations justify the exploration of lower-concentration chlorhexidine preparations that may offer comparable antimicrobial efficacy with fewer adverse effects.

The present study demonstrated a significant difference in effectiveness between alcohol-based handrub and chlorhexidine 2% handwash in reducing bacterial colony counts. The superior reduction observed with chlorhexidine 2% handwash may be attributed to differences in antimicrobial mechanisms and application procedures. Handwashing with chlorhexidine involves mechanical friction and rinsing under running water, which facilitates the removal of adherent microorganisms and prevents re-deposition on the skin surface [1]. This mechanical component likely enhances the antimicrobial action of chlorhexidine, contributing to the greater reduction observed.

Chlorhexidine gluconate in handwash formulations is a positively charged biguanide compound that interacts with negatively charged bacterial cell walls [15]. The electrostatic interaction between chlorhexidine and phosphate-containing components of the bacterial cell envelope enables penetration of chlorhexidine molecules into the cytoplasm, where they exert toxic effects that ultimately lead to microbial cell death [8]. In contrast, alcohol is an amphiphilic compound with both hydrophilic and lipophilic properties, allowing it to penetrate microbial envelopes. Exposure to alcohol disrupts membrane fluidity, destabilizes membrane integrity, and denatures structural proteins, thereby impairing metabolic processes essential for microbial survival [16]. Although both agents are effective, the additional mechanical rinsing in handwashing may explain the superior performance of chlorhexidine 2%.

Bacterial identification before and after hand hygiene revealed the presence of *Bacillus* spp. and coagulase-negative *Staphylococcus* (CoNS) in the handrub group, whereas the handwash group showed *Bacillus* spp., CoNS, and *Stenotrophomonas maltophilia*. *Bacillus* spp. are spore-forming bacteria commonly found on the hands of healthcare workers during clinical activities, and their spores may be transmitted between patients via contaminated hands [17]. CoNS represent normal skin flora but may become clinically significant in immunocompromised hosts, where contamination can lead to invasive infections [18]. *Stenotrophomonas maltophilia* is a Gram-negative bacillus that colonizes the skin, gastrointestinal tract, and respiratory tract, and may serve as a nosocomial pathogen originating from contaminated disinfectants, infusion fluids, or hospital water systems [19].

Normal flora consist of microorganisms that accumulate on healthy skin and mucosal surfaces [20]. These organisms do not cause disease when present in normal quantities [21]. Skin and mucosal flora are categorized into resident and transient groups [22]. Resident flora are specific microorganisms adapted to particular body sites and persist over time, whereas transient flora originate from the environment and do not permanently colonize the skin [21]. Hand hygiene practices using alcohol-based handrub or chlorhexidine handwash influence the composition of detectable bacteria. Transient flora such as *Bacillus* spp. and *Stenotrophomonas maltophilia* are less frequently detected after hand hygiene because they reside on the skin surface and are easily removed through washing activities [23].

This study has several limitations. First, individual skin conditions were not assessed due to the unavailability of a skin analyzer in the Microbiology Laboratory, which may influence the effectiveness of antiseptic agents. Second, population selection could not be standardized across identical clinical units because of limited sample availability in each ward, potentially affecting the generalizability of findings.

CONCLUSION

The study demonstrated that chlorhexidine 2% handwash was significantly more effective than chlorhexidine gluconate 0.5% with 70% ethyl alcohol handrub in reducing bacterial colony counts. The superior effectiveness of handwash is likely influenced by both the antimicrobial action of chlorhexidine and the mechanical rinsing process under running water, which enhances microbial removal.

Ethical consideration, competing interest and source of funding

-Ethical approval for the research was granted by the Health Research Ethics Committee (KEPK) of Sultan Agung Islamic Hospital under approval number 32/KEPK-RSISA/II/2024.
-There is no conflict of interest related to this publication.
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