Local Production of World Health Organization Recommended Hand Rub Formulation and Comparison of its Efficacy with a Commercially Available Alcohol-Based Hand Rub

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Abstract

Objective: To produce a World Health Organization (WHO)-recommended hand rub formulation locally and compare its efficacy with a commercially available Alcohol-based Hand Rub (ABHR) - Sterillium. **Methods:** A non-randomized comparative study was conducted. Sixty healthcare workers were divided into two groups - Group A (WHO-recommended hand rub) and Group B (Sterillium). WHO-recommended hand rub was prepared according to the specified formula. Each participant from study Group A received three ml of WHO-recommended hand rub formulation, and each study Group B participant received three ml of Sterillium. Bacterial samples of each participant (from both hands) were collected before and after using an ABHR solution. Samples were inoculated on the nutrient agar plates and incubated at 37^{0}C for 24 hours. The total number of bacterial colonies grown on plates inoculated before and after ABHR use was counted and identified by standard microbiology techniques and compared between the two groups. The WHO-recommended hand rub and Sterillium were compared and analyzed using differences in proportion. **Results:** The overall reduction in bacterial growth was comparable for both the hand rubs, with an average load reduction of log 1.5607 for locally produced hand rub solution and an average load reduction of log 1.609 in the case of Sterillium. **Conclusion:** Both of the handrubs are comparable in efficacy and are effective against all the bacteria isolated from the palms of HCWs (Coagulase Negative *Staphylococcus* (CoNS), *Micrococcus spp*., *Acinetobacter spp*., *Bacillus spp*., *Pseudomonas spp*., and *Citrobacter*), including Methicillin-Sensitive *Staphylococcus aureus*.

Keywords: Hand Sanitizers, Hand Disinfection, Hand Hygiene

Introduction

Infection transmission through contaminated hands of healthcare workers (HCWs) is a common pattern seen in most healthcare settings[1,2]. Pathogens like Vancomycin-Resistant *Enterococcus* (VRE), Methicillin-Resistant *Staphylococcus aureus* (MRSA), Gram-negative bacilli, *Candida* species, *Influenza* virus, *Clostridium difficile,* etc. can be present on hands of HCWs[3]. Failure to follow proper hand hygiene practices is a prime cause of Healthcare-Associated Infections (HCAIs) and the spread of multidrug-resistant microorganisms. It has been recognized as an important contributor to the outbreaks of infectious diseases by the World Health Organisation (WHO)[4]. The efficacy of hand hygiene practices and the level of sanitation achieved is hugely dependent on the type of disinfecting agent used[5]. Various means of disinfecting the hands exist in the form of traditional soaps, antimicrobial soaps, non-water-dependent Alcohol-Based Hand Rub (ABHR) solutions, gels, and foams. Worldwide, however, two main methods for the maintenance of hand hygiene are followed – Traditional soap and water method is mainly used in the United States, while alcohol-based sanitizers are used in the majority of Europe if visible soiling of hands is not present[6]. WHO has recognized that hand washing by HCWs with soap and water can prevent infection in patients and is thus a potent way to intercept the transmission of HCAIs[4]. Handwashing with soap removes the body's fatty acid from the skin, which is disadvantageous as it may cause cracks in the skin, which will provide an entry portal for pathogens[7,8]. In contrast, skincare products such as emollients are added to the antibacterial composition of high-quality hand disinfectants. ABHR can be made readily available at the bedside, are time-effective, do not require the use of water, and hence are easy and uncomplicated to use[9].

Several commercial hand rub solutions are available for hand hygiene, but most are expensive. In a developing country like India, effective and economical hand rub solutions are the need of the day. The availability of locally produced, effective, and economically cheaper hand rub solutions in all healthcare settings will increase the convenience and frequency of hand hygiene practices, which will cause a reduction in infection[4].

With this background, this non-randomized comparative study was conducted to prepare a WHO-recommended alcohol-based hand rub locally and compare its efficacy with a commercially available ABHR.

Objectives

This study aimed to compare locally prepared WHO-recommended formulation with a commercially available ABHR.

The objectives were:

1) Local production of WHO-recommended Isopropyl ABHR formulation followed by post-production alcohol concentration analysis

2) To compare the efficacy of the locally prepared hand rub formulation with a commercially available ABHR

3) To identify the bacterial population on HCWs hands and determine the Log reduction in bacterial colony counts on HCWs' hands after the application of hand rub solutions

Materials and Methods

This was a non-randomized comparative study conducted in a tertiary care hospital from 1st April 2021 to 31st May 2021. The inclusion criteria was Health Care workers (HCWs) between the ages of 18 to 65, not having any skin damage and skin disorders affecting the hands, or any known allergies to ABHR. A total of 60 HCWs, including doctors and nurses, were randomly selected from wards.

The study was conducted using the following steps:

Step One: Local preparation of WHO-recommended Hand rub formulation[4]

The details of the reagents used for preparation are displayed in Table 1.

Table 1: Reagents used for the preparation of WHO-recommended Hand Rub solution

Sr. No .	Reagent used	Concentration	Amount
1.	Isopropyl alcohol (Merck Pvt. Ltd, Mumbai, India)	99.8%	751.5 ml
2.	Hydrogen Peroxide (Merck Pvt. Ltd, Mumbai, India)	3%	41.7 ml
3.	Glycerol (Merck Pvt. Ltd, Mumbai, India)	98%	14.5 ml
4.	Distilled water	-	192.3 ml

Procedure

The solution was prepared with all aseptic precautions in a dedicated, cool, dry, well-ventilated room away from sunlight.

To prepare 1000 ml of solution:

1) 750 ml of isopropyl alcohol 99.8% was dispensed in a 1000 ml cylinder.
2) Using a 5 ml micropipette, 1.5 ml more of isopropyl alcohol was added to the same cylinder.
3) Using a 50 ml measuring cylinder, 41.7 ml of 3% hydrogen peroxide was added to a cylinder containing isopropyl alcohol.
4) Using a 10 ml micropipette, 14.5 ml of 98% glycerol was added to a cylinder containing isopropyl alcohol.
5) Finally, 192.3 ml of cooled distilled water was added to make 1000 ml of the formulation.
6) A 1000 ml cylinder was gently shaken to mix contents.
7) The final target content of formulation expected was:
   Isopropyl alcohol – ≥75% v/v
   Glycerol – 1.45% v/v
   Hydrogen peroxide – 0.125% v/v
8) Immediately, the solution was divided into two 500 ml plastic bottles and placed in quarantine for 72 hours.

   The alcohol concentration of the formulation produced was tested using an alcoholmeter calibrated for ethanol at 25^{0}C to ensure standard production. The locally produced formulation was also subjected to a sterility check before use by inoculating on sterile nutrient agar and incubating at 37^{0}C for 24 hours.

Step Two: Testing efficacy of the formulation prepared in Step One on HCWs (Study group A - 30 participants and testing efficacy of commercially available ABHR (Study group B - 30 participants)

It was conducted as per the guidelines adopted from a similar study conducted by Shetty et al.[10]. Identification of bacterial population on HCWs hands during testing of efficacy.

HCWs in wards and critical units on unannounced study dates were approached for participation. A total of 60 HCWs were enrolled in the study. They were equally divided into Study Group A and Study Group B. Each group consisted of 15 doctors and 15 nursing staff. After taking written informed consent, each member of Group A was given a recommended amount of three ml of locally prepared WHO recommended Hand rub formulation, and Group B members received 3 ml of Sterillium (Raman and Weil Pvt. Ltd., Mumbai, India).

A total of four swabs were collected from each participant. Two swabs (one from each hand) were collected before the hand rub application. A sterile swab moistened with sterile saline was rolled over each hand's palm, fingers, and web space. After the swab collection, participants were asked to perform hand hygiene as per WHO's six steps of hand hygiene. Hands were allowed to completely dry in the air, and a repeat sample was collected with swabs (one from each hand). Swabs were properly labeled and transported to the microbiology laboratory. All the study samples were collected within one month of the preparation of the hand rub.

Each swab was inoculated on one nutrient agar plate. The swab was rolled three times on the center of the petri dish. With a sterile inoculating loop, agar was streaked perpendicular to the inoculation line all over the plate. The nutrient agar plates were incubated at 37^{0}C for 24 hours. The total number of bacterial colonies that were grown were counted. Bacterial colonies were identified by standard microbiology techniques[11]. The number of bacterial colonies isolated from both hands before the application of hand rub was added up, and the number of bacterial colonies isolated from both hands after the application of hand rub was added up. The colony count was later compared to look for Log reduction in colony count. Details were noted in the Case Study Form.

The commercially prepared ABHR used here was Sterillium (Raman and Weil Pvt. Ltd., Mumbai, India). The composition of Sterillium solution per 100 grams includes 2-propanol 45.0 grams, 1- propanol 30 grams, Mecetronium methyl sulfate 0.2 grams, emollients, skin-ting substances[9].

Result

The concentration of the WHO-recommended Hand rub formulation achieved was 77% when tested using an alcoholmeter calibrated for ethanol at 25^{0}C. Since we had used isopropyl alcohol instead of ethanol for manufacturing sanitizer, the corrected value of concentration is 75% v/v, which is within the target range[4].

Bacterial growth was observed in all 60 (100%) specimens tested before the application of the hand rub (Figure 1). Bacteria isolated from Group A members, in descending order, were Coagulase Negative Staphylococci (CoNS), *Micrococcus spp., Acinetobacter spp.,* Methicillin-Sensitive *Staphylococcus aureus* (MSSA), *Bacillus spp., Pseudomonas spp., and Citrobacter*.

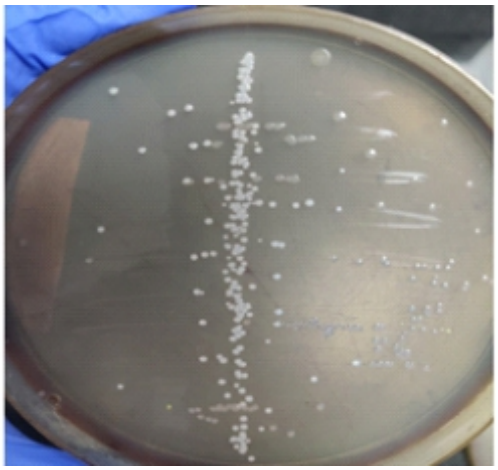

Figure 1: Agar plate showing the growth of Colony Forming Units before the use of locally produced WHO-recommended Hand Rub formulation

A total of 1382 Colony Forming Unit (CFU) were isolated from 30 individuals before WHO recommended hand rub use and only 38 CFU after use, as shown in Table 2 and Figure 2. A load reduction of Log 1.5607, corresponding to a 97.25% reduction in CFU, was observed after WHO-recommended hand rub use.

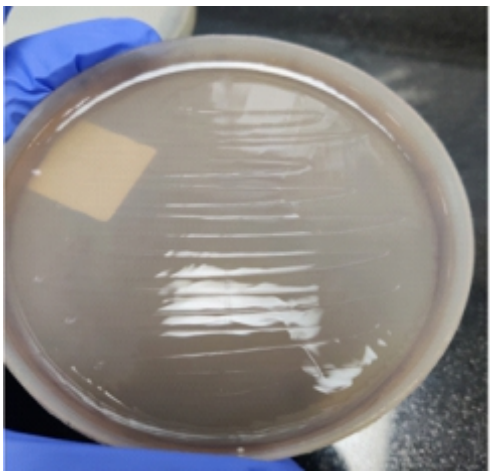

Figure 2: Agar plate showing a significant reduction in the growth of Colony Forming Units after the use of locally produced WHO-recommended Hand Rub formulation

Table 2: **Micro-organisms isolated from the hands of group A members**

Sr. No.	Isolates grown	Before locally produced WHO-recommended hand rub formulation use	After locally produced WHO-recommended hand rub formulation use	Percentage reduction	Log reduction
1	*Coagulase negative Staphylococci* (CoNS)	748	29	96.12 %	Log 1.411
2	*Acinetobacter spp.*	453	9	98.01 %	Log 1.702
3	*Micrococcus spp.*	97	0	~99.99%	Log 3
4	*Bacillus spp.*	55	0	~99.99%	Log 3
5	*Pseudomonas spp.*	18	0	~99.99%	Log 3
6	Methicillin-Sensitive Staphylococcus *aureus*	11	0	~99.99%	Log 3
Total		**1382**	**38**	**97.25%**	**Log 1.560**

The bacteria isolated from group B members, in descending order of prevalence, were Coagulase-Negative-Staphylococci (CoNS), *Acinetobacter spp.*, *Micrococcus spp.*, *Bacillus spp., Pseudomonas spp*., Methicillin-Sensitive *Staphylococcus aureus*. A total of 1179 colonies were isolated before the use of Sterillium (Raman and Weil Pvt. Ltd., Mumbai, India), and 29 colonies were seen after use, as shown in Table 3. A load reduction of Log 1.609, corresponding to a percentage reduction of 97.54% in CFUs, was observed.

Table 3: **Micro-organisms isolated from the hands of group B members**

Sr. No.	Isolates grown	Sterillium (before use)	Sterillium (after use)	Percentage reduction	Log reduction
1	Coagulase-Negative-Staphylococci (CoNS)	522	14	97.31%	Log 1.571
2	*Micrococcus species*	313	0	~99.99%	Log 3
3	*Acinetobacter*	193	13	93.78%	Log 1.171
4	Methicillin-Sensitive Staphylococcus aureus	71	0	99.99%	Log 3
5	*Bacillus species*	55	2	96.36%	Log 1.439
6	*Pseudomonas spp.*	19	0	~99.99%	Log 3
7	*Citrobacter spp.*	6	0	~99.99%	Log 3
Total		**1179**	**29**	**97.54%**	**Log 1.609**

It was also observed that both the hand rub solutions had an inhibitory effect on all the types of bacteria isolated.

The overall reduction in bacterial growth after using hand rub solution compared to bacterial growth before using hand rub solution ranged from Log 1.17 to Log 3 for Sterillium (Raman and Weil Pvt. Ltd., Mumbai, India) and Log 1.41 to Log 3 for WHO-recommended Hand rub formulation. A comparable reduction of Log 1.5607 and Log 1.609 was obtained for in-house locally produced WHO- recommended hand rub and Sterillium when taking into account all specimens.

Complete inhibition of bacterial growth is seen in 25 study participants who used Sterillium (Raman and Weil Pvt. Ltd., Mumbai, India) and in 25 who used WHO-recommended Hand rub formulation. In terms of percentage calculation, 2.74% of colonies {(38 x 100)÷1382} were left after WHO-recommended hand rub solution use, while 2.459% of colonies {(29 x 100)÷1179} were left after Sterillium use. These percentages are statistically not significant, with a p-value of 0.68 for a 5% level of significance, indicating that the hand rubs are comparable in efficacy.

Discussion

Over the years, hand hygiene has proven to be the basis of the framework for the prevention of healthcare-associated infections. It is a simple intervention that not only reduces contamination of hands by microorganisms[12,13] but also dramatically reduces the rate of nosocomial infections[9,14] and hence decreases the hospital cost due to nosocomial infection-related extended patient stay[15-17]. However, the

compliance rate of handwashing using soap and water is significantly lower[18,19] than the WHO recommended rate of 60%[20]. This could be due to the lack of availability of washbasins in the infrastructure and also due to the inconvenience caused by extended loss of time during patient care, the distance of washbasins from the bedside, and the additional need to dry hands using clean paper towels. Moreover, even when conventional handwashing is practiced, the recommended 30 seconds to one-minute duration is followed in only 35% of the instances[21]. ABHR has proven to be a promising alternative to traditional handwashing due to its time efficiency, availability at the point of care, convenience, simpler mode of application, wider microbiological spectrum, and long shelf life of up to 19 months[4,7,9,21-23].

To conduct our study, we needed to compare locally produced WHO-recommended hand rub formulation to a commercially available hand rub solution. We chose Sterillium due to its common use in hospitals and easy availability. It is noteworthy that Sterillium has been taken as a comparison standard in other studies[24]. It has also been shown to have a higher efficiency against microbes like *Staphylococcus. aureus, Escherichia coli, and Klebsiella spp*. in addition to being significantly better than other ABHR in the test, with a $P < 0.001$[24].

WHO guidelines on the preparation of ABHR solution, have provided instructions with two formulations: 80% v/v Ethanol and 75% v/v Isopropyl Alcohol. Although a larger number of studies have been conducted with formulation-1 (80% v/v ethanol) than with formulation-2 (75% isopropyl alcohol), isopropyl alcohol was selected over ethanol for this study since isopropyl alcohol has greater lipophilicity than ethanol, which renders it more effective against microbes[6].

The concentration of alcohol achieved with the locally produced WHO recommended Hand rub formulation was found to be 77% when tested using an alcoholmeter calibrated for ethanol at 25^0C. It is worth noting that the actual concentration of isopropyl alcohol in the hand rub formulation is slightly less than the reading noted by the ethanol-calibrated. The locally produced formulation hence has a 75% v/v concentration of isopropyl alcohol[4]. This falls within the recommended range of 75% and Standard Deviation (SD) of 85%±5%[4].

In the second step of the study, the efficacy of the locally produced hand rub and Sterillium was tested. Three ml of ABHR was dispensed in each participant's hands as recommended by WHO guidelines. A study by Shetty et al.[10] used two ml for locally produced hand rub formulation and three ml for Sterillium. This difference in the volume dispensed by the author in the study could be to achieve the stipulated rub time of 30 seconds. However, it has been observed that with a decrease in the dispensed amount of ABHR, the efficacy of the hand rub decreases[25].

The microorganisms isolated in Step two of the study were Coagulase-Negative-Staphylococci (CoNS) *spp*, *Acinetobacter spp*, *Micrococcus, Bacillus spp*, *Pseudomonas spp*, and Methicillin-Sensitive *Staphylococcus aureus.* This finding was similar to the microorganisms found in health care settings in different studies conducted over the years[26].

The reduction in bacterial growth was comparable for both, locally produced WHO-recommended hand rub formulation and Sterillium. This comparable efficacy was also found in the study conducted by Shetty et al.[10,27,28]. The overall reduction in bacterial growth after using hand rub solution compared to bacterial growth before using hand rub solution was Log 1.609 (or 97.54%) in the case of Sterillium and Log 1.5607 (or 97.25%) in the case of locally produced hand rub solution. This documented efficacy was considerably higher when compared to the study of Shetty et al.[10] but was comparable to the study conducted in Uganda to compare the anti-microbial efficacy of commercially available hand rub to locally produced alcohol-based hand sanitizer[28].

The comparable efficacy achieved between the two hand rubs being studied was an expected result since the concentration of alcohol, which is the active ingredient of ABHR solution, is comparable (75%) in both solutions. WHO-recommended hand rub solution consisted only of isopropyl alcohol, while Sterillium had a mixed composition of isopropyl alcohol and n-propyl alcohol (1-propyl alcohol). A prior study has noted a higher bactericidal efficacy of n-propyl alcohol over isopropyl alcohol[6]. This could explain the slightly better performance of Sterillium over locally produced hand rub solution.

The study by Shetty et al. noted a reduction in bacterial count in the range from 30.15% - 100% for Sterillium and 33.91% - 100% for locally produced WHO-recommended hand rub formulation, which is lower when compared to our findings. This could be due to the difference in the amount of sanitizer dispensed per participant (Two ml/ person) in the two studies. The higher efficacy achieved in our study could also have been due to the stringent following of WHOs six steps of hand hygiene and higher time of contact with ABHR, as more than 30 seconds is required for drying of alcohol when the recommended volume of 3 ml of ABHR is taken[25].

Given its efficacy, tolerability, and acceptability WHO recommended hand rub solution may be a huge leap in hand hygiene promotion. Its low cost and easy manufacturing process are a virtue that will ensure consistent availability in healthcare settings. As per the cost calculation by WHO, its cost can go as low as US$ 0.30 in Bangladesh and US$ 0.44 in Pakistan per 100 ml[12]. WHO-recommended hand rub has the potential to subjugate the economic barriers to hand hygiene in low- and middle-income countries[29,30].

The study has yielded remarkable and promising results, but there are certain limitations to our study. The study has not ventured into the economics of the production of WHO-recommended hand rub formulation. Further studies can be conducted with locally resourced raw materials to determine the cost of production. If they are economical, they can be implemented at hospital levels to ensure a continuous supply of hand rubs.

Conclusion

WHO Recommended hand rub solution was found to be comparable in efficacy to Sterillium (p=0.68) with a significant bacterial reduction rate. It was found to be effective against all the bacteria isolated from the hands of HCWs including Methicillin-Sensitive *Staphylococcus aureus*. Demonstrating the reliable efficacy of the WHO-recommended Hand rub formula has opened avenues for other studies exploring the cost-effectiveness of the hand rub. This study may prove to be a significant benchmark in the field of hand hygiene as it validates the efficacy of the WHO-recommended Hand rub formula which has the potential which can bridge the economic hindrance to hand hygiene in low- and middle-income countries.

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Conflict of Interest: Nil

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Ethical consideration

This study was approved by Institutional Ethics Committee by letter No. BVDUMC/IEC/05 dated 8th May 2020. All the participants were explained the nature of the study and the possibility of publication of results while being enrolled in the study. Written informed consent was obtained from all participants. It was an academic trial. As per new drugs and clinical trials rules 2019, registration with Clinical Trials Registry-India (CTRI) was exempted.

Authors' Contribution

FNUS: Data collection, implementation, data analysis, interpretation, and manuscript writing; AM: conceptualization, design, data collection, implementation, data analysis, interpretation, and manuscript writing; AK: data collection, implementation, data analysis, interpretation, and manuscript writing; MK: conceptualization, design, data collection, implementation, data analysis, interpretation, and manuscript writing; AT: conceptualization, design, data collection, implementation, data analysis, interpretation, and manuscript writing; SD: design, implementation, data analysis, interpretation, and manuscript writing; MM: conceptualization, design, data analysis, interpretation, and manuscript writing

Data availability statement

Data will be available with corresponding author on request.

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