

Antimicrobial activity of a WHO ethanol-based hand rub solution in a hospital setting

Nina Dwi Putri¹, Hindra Irawan Satari¹, Mulya Rahma Karyanti¹, Ari Prayitno¹, Pratama Wicaksana¹, Anis Karuniawati², Delly Chipta Lestari², Nabila Maudy Salma¹, Shindy Claudya Aprianti¹, Amalia Almira¹, Andi Annisa Rusyda Khafiyani¹

Abstract

Background Hand hygiene is essential in reducing healthcare-associated infections. Alcohol-based hand rub solutions have been reported to have superior antimicrobial efficacy on both bacteria and lipophilic viruses compared to washing with hand soap. In low- and middle-income countries, the cost of infection control poses a challenge. Our hospital produced an ethanol-based hand rub based on a WHO formulation to reduce the infection prevention costs.

Objectives To identify the antimicrobial activity of a WHO ethanol-based hand rub solution against bacterial contamination on the hands of healthcare workers at the Department of Child Health, Dr. Cipto Mangunkusumo National Referral Hospital.

Methods This cross-sectional study was performed on the hands of healthcare workers (physicians and nurses) working in the Department of Child Health, Dr. Cipto Mangunkusumo National Referral Hospital, Jakarta. A total of 225 specimens from 75 subjects were obtained by collecting swabs on both hands before and after participants worked in the Department of Child Health, Dr. Cipto Mangunkusumo General Hospital. Bacterial culture tests were performed to identify Gram positive and Gram-negative bacteria. Bacteria were grouped into no-growth/*Bacillus sp.*, *Enterobacteriaceae*, cocci, and non-fermenter groups.

Results The use of our WHO ethanol-based hand rub solution, generally resulted in a statistically significant decrease in bacterial growth from 84 to 54.6%, before compared to after the hand rub was performed. In more detail, there was a 72.7% decrease in *Enterobacteriaceae*, a 71.4% decrease in non-fermenters, an 8.6% decrease in cocci and a 44.1% increase in the number of specimens showing no growth bacteria/*Bacillus sp.*

Conclusion Our WHO ethanol-based hand rub has significant antimicrobial activity for common nosocomial pathogens (e.g., *Staphylococcus aureus*, *P. aeruginosa*, and *K. pneumoniae*). [Paediatr Indones. 2022;62:222-6 DOI: 10.14238/pi62.4.2022.232-6].

Keywords: hand hygiene; ethanol-based; hand rub; WHO

Hand hygiene has been established as a vital component in preventing hospital-associated infections (HAIs). Several studies reported that alcohol-based hand rub solutions have superior antimicrobial efficacy and are associated with higher hand hygiene compliance in healthcare workers (HCWs) compared to washing with hand soap.^{1,2} Alcohol-based hand antiseptic solutions contain either ethanol, isopropanol or n-propanol, or a combination of these agents. The antimicrobial activity of alcohol results from its ability to denature proteins. It has high germicidal activity against Gram positive and Gram negative bacteria in vitro. However, it has zero potential activity against bacterial spores, protozoan oocysts, and non-lipophilic viruses.³ Alcohols are rapidly germicidal when applied to the skin, but residual activity is not persistent. Thus, they are usually combined with humectants (e.g., glycerin) and preservatives to achieve prolonged activity against transient pathogens.⁴ The germicidal

From the Department of Child Health¹ and Department of Microbiology², Universitas Indonesia Medical School/Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia.

Corresponding author: Nina Dwi Putri. Infectious and Tropical Pediatric Division, Department of Child Health, Universitas Indonesia Medical School/Dr. Cipto Mangunkusumo General Hospital, Jl. Diponegoro No. 71, Jakarta 10430, Indonesia. Email: ninadwip@gmail.com

Submitted October 23, 2021. Accepted July 25, 2022.

effect depends on the solution's concentration, with solutions containing 60-95% alcohol having the best germicidal effect; 70-80% alcohol could inactivate HIV and hepatitis B virus in 2-10 minutes.⁵

A systematic review on the effectiveness of alcohol-based hand hygiene solutions showed that alcohol-based hand rubs removed microorganisms more effectively, required less time for microorganism elimination, and caused minimal irritation to the skin compared to handwashing soap or other antiseptic agents.⁴ However, further study is needed to evaluate its efficacy and effects on microorganism transmission in the hospital. We aimed to evaluate the antimicrobial effect of an alcohol-based hand rub solution produced at Dr. Cipto Mangunkusumo National Referral Hospital, Jakarta.

Methods

This cross-sectional study was conducted between July and November 2014 in the Department Child Health, Dr. Cipto Mangunkusumo National Referral Hospital, Jakarta, Indonesia. Seventy-five healthcare workers were enrolled consecutively, including nurses and physicians working in the neonatal intensive care unit (NICU), pediatric intensive care unit (PICU), and pediatric inpatient ward, who worked in direct contact with patients and in environments where the risk of microbial transmission was high. All enrolled healthcare workers had both of their hands swabbed on three separate occasions: (1) after interaction with the environment and before performing the hand hygiene procedure (cleaning their hands using hand rub solution); (2) after performing the hand hygiene procedure; and (3) after contact with patients. In the hospital environment, healthcare workers were obliged to comply with Dr. Cipto Mangunkusumo National Referral Hospital standards on hand hygiene using the hand rub.

Specimens were collected from the surfaces of fingers and palms using sterile cotton swabs pre-moistened with sterile saline solution and immediately transported to the laboratory. Specimens were then inoculated on thioglycolate liquid medium, sheep blood agar (SBA), and MacConkey's agar, and incubated for 24-48 hours at 35-37°C. Colony growth and morphology were monitored for each sample. Gram

positive and Gram negative bacteria were identified using standard microbiological procedures.

The hand rub solution used in this study was an alcohol-based hand sanitizer solution produced in-house according to the *World Health Organization* (WHO) formulation, containing 96% ethanol (83.33 mL for every 100 mL of solution), 3% H₂O₂, glycerin, and water.⁶ The production cost for every 500 mL of the hand rub solution was IDR 36,874 (approximately USD 2.62). As per hospital standards, all subjects used this hand rub following the hand hygiene procedure, i.e., washing hands at the five specified moments using the six steps of handwashing, with a minimum duration of 20-30 seconds.⁴

All analyses were performed using *IBM SPSS 20.0 for Windows*. Bacteria on hands are presented descriptively in text and tables. Statistical analyses of variables (no growth/*Bacillus sp.*, cocci, non-fermenters, and *Enterobacteriaceae*) were performed using Friedman's test and the marginal homogeneity test. This study was approved by the Research Ethics Committee, Faculty of Medicine, Universitas Indonesia.

Results

We obtained a total of 225 hand swab specimens from 25 HCWs at each pediatric unit. Of specimens taken before performing the hand hygiene procedures, 84% showed colony growth, vs. 54.6% of specimens taken after performing hand hygiene procedures. The observed patterns of microorganism growth are presented in **Table 1**. We grouped bacteria into four major categories: no growth/*Bacillus sp.* (since *Bacillus sp.* is considered to be resident flora), cocci, non-fermenters (consisting of bacteria commonly found in the environment), and *Enterobacteriaceae* (consisting of bacteria frequently found colonizing the gastrointestinal tract of feces).

When the swabs taken before and after hand rub use were compared, there was a 44.1% increase in the number of specimens showing no growth bacteria/*Bacillus sp.*, and a 72.7%, 71.4%, and 8.6% decrease in the number of specimens showing growth of *Enterobacteriaceae*, non-fermenters, and cocci, respectively ($P < 0.001$) (**Table 2**).

There was a 51% decrease in no growth bacteria/*Bacillus sp.* of specimens taken after patient contact

Table 1. Microorganism patterns from swab specimens obtained from HCWs in the Pediatrics Department of Dr. Cipto Mangunkusumo National Referral Hospital, July – November 2014 before and after performing hand rub, and after patient contact

Microorganism	Before performing hand rub (n=25)	After hand rub (n=25)	After patient contact (n=25)
PICU, n			
No growth	7	14	4
Growth	18	11	21
<i>Bacillus</i> sp.	2	3	2
<i>Acinetobacter baumannii</i>	1	0	1
<i>Acinetobacter lwoffii</i>	0	1	0
<i>Burkholderia cepacia</i>	2	0	0
<i>Enterobacter cloacae</i>	1	0	1
<i>Pseudomonas stutzeri</i>	1	0	0
<i>Serratia marcescens</i>	1	0	2
<i>Serratia odorifera</i>	1	0	0
<i>Sphingomonas paucimobilis</i>	2	0	0
<i>Staphylococcus aureus</i>	1	1	0
<i>Staphylococcus epidermidis</i>	6	6	9
<i>Staphylococcus haemolyticus</i>	0	0	1
<i>Enterobacter aerogenes</i>	0	0	1
<i>Pseudomonas aeruginosa</i>	0	0	2
<i>Pantoea</i> sp.	0	0	2
Pediatric ward, n			
No growth	3	7	1
Growth	22	18	24
<i>Bacillus</i> sp.	13	9	11
<i>Acinetobacter baumannii</i>	1	0	0
<i>Pseudomonas aeruginosa</i>	0	1	0
<i>Enterobacter cloacae</i>	1	0	2
<i>Staphylococcus aureus</i>	2	2	2
<i>Staphylococcus epidermidis</i> (incl. MRSE)	5	6	6
<i>Klebsiella pneumoniae</i>	0	0	1
<i>Burkholderia cepacia</i>	0	0	1
<i>Pseudomonas stutzeri</i>	0	0	1
NICU, n			
No growth	2	13	2
Growth	23	12	23
<i>Bacillus</i> sp.	7	3	4
<i>Aerococcus viridans</i>	1	0	0
<i>Enterobacter aerogenes</i>	1	0	1
<i>Klebsiella pneumoniae</i>	1	2	1
<i>Proteus mirabilis</i>	0	1	1
<i>Pantoea</i> sp.	4	0	1
<i>Serratia odorifera</i>	1	0	0
<i>Staphylococcus aureus</i>	3	2	3
<i>Staphylococcus epidermidis</i> (incl. MRSE)	4	4	8
<i>Streptococcus viridans, alpha-hem.</i>	1	0	0
<i>Acinetobacter baumannii</i>	0	0	2
<i>Enterococcus faecium</i>	0	0	1
<i>Serratia fonticola</i>	0	0	1

compared to after hand rub. In contrast, the number of non-fermenter-, cocci-, and *Enterobacteriaceae*-containing specimens significantly increased (5 (250%) 9 (42.8%), and 11 (366.6%), respectively). These results were statistically significant (Table 3).

Discussion

In hospital settings, the four major determinants for choice of hand hygiene agents are: (1) antimicrobial profile; (2) acceptance of usage; (3) cost; and (4)

Table 2. Microorganism distribution at specific times

Microorganism groups	Before hand rub and after exposure to environment (n=75)	After hand rub (n=75)	After patient contact (n=75)	P value
No growth/ <i>Bacillus sp.</i> , n (%)	34 (45.3)	49 (65.3)	24 (32)	<0.001
Cocci, n(%)	23 (30.6)	21 (28)	30 (40)	
Non-fermenters, n(%)	7 (9.3)	2 (2.6)	7 (9.3)	
<i>Enterobacteriaceae</i> , n(%)	11 (14.6)	3 (4)	14 (18.6)	

*Friedman test

Table 3. Post-hoc analysis of different distribution of microorganisms in the Department of Child Health

Microorganism groups, n (%)	Before hand rub (n=75)	After hand rub (n=75)	After patient contact (n=75)	P value
No growth/ <i>Bacillus sp.</i>	34 (45.3)	49 (65.3)		<0.001
Cocci	23 (30.6)	21 (28)		
Non-fermenter	7 (9.3)	2 (2.6)		
<i>Enterobacteriaceae</i>	11 (14.6)	3 (4)		
No growth/ <i>Bacillus sp.</i>		49 (65.3)	24 (32)	<0.001
Cocci		21 (28)	30 (40)	
Non-fermenter		2 (2.6)	7 (9.3)	
<i>Enterobacteriaceae</i>		3 (4)	14 (18.6)	

bactericidal, fungicidal, and viricidal activity, especially in intensive care areas, which are usually reported to have the highest rates of cross-transmission.⁷ We evaluated microorganisms on the hands of HCWs before and after alcohol-based (95% ethanol) hand rub use and found an association between the use of ethanol-based hand rub solution and the types of microorganisms present on swabs from the hands of HCW. We noted a significant decrease in the number of HCWs who tested positive and variation of bacterial colonization after hand rub use compared to before hand hygiene procedures were performed.

This ethanol-based hand rub was most effective in decreasing *Enterobacteriaceae* (including *Enterobacter aerogenes*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Pantoea sp.*, *Serratia marcescens*, *Serratia odorifera*, *Serratia fonticola*, and *Proteus mirabilis*), followed by non-fermenter bacteria (including *Acinetobacter baumannii*, *Acinetobacter iwoffii*, *Burkholderia cepacia*, *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, and *Sphingomonas paucimobilis*), and the cocci group (including *Aerococcus viridans*, *Staphylococcus aureus*, CoNS, *Streptococcus viridans* and other alpha-hemolytic streptococcus). These findings were in agreement with a Russian NICU study, where a significant decrease in nosocomial colonization of *Klebsiella pneumoniae* was found after usage of alcohol-based antiseptics.³ A previous study evaluating the bactericidal activity of 85% ethanol hand

gel showed a similar result. It reduced the levels of all Gram positive bacteria (including *S. aureus* and CoNS) and Gram negative bacteria (including *A. baumannii*, *A. iwoffii*, *B. cepacia*, *E. aerogenes*, *E. cloacae*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, and *S. marcescens*) within 15 seconds, with a reduction factor (RF) of >5.⁸ When compared to other alcohol-based sanitizers (isopropyl alcohol, denatured alcohol, and ethyl alcohol), ethanol showed superior inhibitory activity on all tested organisms (*E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, and *S. pneumoniae*).⁹ Another study evaluating the antibacterial activity of widely used alcohol-based hand sanitizers on common pathogenic bacteria found that ethanol reduced the bacterial load by 98%, with the largest zones of inhibition for methicillin-resistance *S. aureus* (MRSA), *S. aureus*, CoNS, and *S. typhi* (24 mm, 23 mm, 24 mm, and 20 mm, respectively).¹⁰ The percentages of growth and no growth in our study suggest that alcohol-based hand rub effectively decreases colonization of microorganisms, yet it is also evident that microorganism levels increased after patient contact.

In conclusion, the WHO ethanol-based hand rub solution produced in-house at Dr. Cipto Mangunkusumo National Referral Hospital shows significant antimicrobial activity for common nosocomial pathogens such as *Staphylococcus aureus*, *P. aeruginosa*, *K. pneumoniae*, *Enterobacter sp.*, and others.

Conflicts of interest

None declared.

Funding acknowledgment

The authors received no specific grants from any funding agency in the public, commercial, or not-for-profit sectors.

References

1. Widmer AF, Dangel M. Alcohol-based handrub: evaluation of technique and microbiological efficacy with international infection control professionals. *Infect Control Hosp Epidemiol*. 2004;25:207-9. DOI: 10.1086/502379.
2. Laustsen S, Lund E, Bibby BM, Kristensen B, Thulstrup AM, Kjolseth-Moller J. Effect of correctly using alcohol-based hand rub in a clinical setting. *Infect Control Hosp Epidemiol*. 2008;29:954-6. DOI: 10.1086/590393.
3. Brown SM, Lubimova AV, Khrustalyeva NM, Shulaeva SV, Tekhova I, Zueva LP, et al. Use of an alcohol-based handrub and quality improvement interventions to improve hand hygiene in a Russian neonatal intensive care unit. *Infect Control Hosp Epidemiol*. 2003;24:172-9. DOI: 10.1086/502186.
4. World Health Organization guidelines on hand hygiene in health care [Internet]. WHO Int. 2021 [cited 2021 September 8]. Available from: <https://www.who.int/publications/i/item/9789241597906>.
5. Padsalg A, Jain D, Bidkar S, Harinarayana D, Jadhav V. Preparation and evaluation of handrub disinfectant. *Asian J Pharm*. 2008;2:18-21. DOI: 10.4103/0973-8398.41559.
6. Guide to local production: WHO-recommended handrub formulations. [Internet]. WHO.Int. 2021 [cited 2021 September 8]. Available from: https://www.who.int/gpsc/5may/Guide_to_Local_Production.pdf.
7. Mathur P. Hand hygiene: back to the basics of infection control. *Indian J Med Res*. 2011;134:611-20. DOI: 10.4103/0971-5916.90985.
8. Kampf G, Hollingsworth A. Comprehensive bactericidal activity of an ethanol-based hand gel in 15 seconds. *Ann Clin Microbiol Antimicrob*. 2008;7:2. DOI: 10.1186/1476-0711-7-2.
9. Oke MA, Bello AB, Odebisi MB, El-Imam AMA, Kazeem MO. Evaluation of antibacterial efficacy of some alcohol-based hand sanitizers sold in Ilorin (North-Central Nigeria). *Ife J Sci*. 2013;15:111-7.
10. Kumar D, Kaushal SK, Kumar G, Prakash V, Prakash P, Nath G. Evaluation of the antibacterial activity of commonly used alcohol-based hand sanitizers on common pathogenic bacteria. *Indian J Appl Res*. 2015;3:2249-555. DOI: 10.36106/ijar.