



Alcohol in gel is as effective as chlorhexidine in the reduction of microbiota on hands in hospital nurses

[El alcohol en gel es tan eficaz como la clorhexidina en la reducción de la microbiota de las manos en enfermeras de hospital]

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Abstract

Context: Alcohol gel (70% ethanol, AG) and 2% chlorhexidine foam (CF) are hand antiseptics recommended by the World Health Organization. Although both are effective, it is unclear if one is superior to the other in a hospital setting.

Aims: To evaluate the disinfectant ability comparing AG and CF by analyzing microbial reduction on the hands of nursing staff from two hospitals in Northern Peru.

Methods: The nursing staff was randomly assigned to the AG and CF treatment groups. Before and after antiseptics, samples were taken by inserting the hands into a sterile self-closing bag containing peptone water. A sample of the liquid medium was plated on agar and used to calculate the CFU/mL of the peptone water. Participant data was analyzed in aggregate or according to hospital, profession, or service.

Results: Both AG and CF were significantly effective in reducing CFU/mL. In aggregate, CFU/mL reduction was 80.9% for AG and 91.3% for CF, but the difference was not statistically significant. However, a small statistically significant difference in favor of CF was observed in the surgical center of both hospitals. These results are confirmed with multivariate analysis.

Conclusions: AG and CF are both effective in hand antiseptics, but other factors are important when deciding which agent is appropriate for a specific healthcare setting.

Keywords: local anti-infective agents; chlorhexidine; ethanol; hand disinfection; hygiene; microbiota.

Resumen

Contexto: El alcohol en gel (etanol al 70%, AG) y la espuma de clorhexidina al 2% (CF) son antisépticos de manos recomendados por la Organización Mundial de la Salud. Aunque ambos son eficaces, no está claro si uno es superior al otro en un entorno hospitalario.

Objetivos: Evaluar la capacidad desinfectante comparando AG y CF mediante el análisis de la reducción microbiana en las manos del personal de enfermería de dos hospitales del norte de Perú.

Métodos: El personal de enfermería fue asignado aleatoriamente a los grupos de tratamiento AG y CF. Antes y después de la antiseptia, se tomaron muestras introduciendo las manos en una bolsa estéril de cierre automático que contenía agua de peptona. Una muestra del medio líquido se sembró en agar y se utilizó para calcular las UFC/mL del agua de peptona. Los datos de los participantes se analizaron en conjunto o según el hospital, la profesión o el servicio.

Resultados: Tanto AG como CF fueron significativamente eficaces en la reducción de UFC/mL. En conjunto, la reducción de UFC/mL fue del 80,9% para AG y del 91,3% para CF, pero la diferencia no fue estadísticamente significativa. Sin embargo, se observó una pequeña diferencia estadísticamente significativa a favor de la CF en el centro quirúrgico de ambos hospitales. Estos resultados se confirman con el análisis multivariante.

Conclusiones: Tanto AG como CF son eficaces en la antiseptia de manos, pero otros factores son importantes a la hora de decidir qué agente es apropiado para un entorno sanitario específico.

Palabras Clave: agentes antiinfecciosos locales; clorhexidina; desinfección de manos; etanol; higiene; microbiota.

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INTRODUCTION

During hospitalization, patients may be exposed to a variety of disease-causing microbes, which can lead to hospital-acquired infections (HAI). It has been well established that HAI are a result of microbial transmission by fomite or directly between patients. The most common form of direct and indirect nosocomial transmission of pathogens is by hand contact and transferring an inoculum of a pathogen to a new surface or host (Diomedei et al., 2017; Gold et al., 2022; Sosa, 2019).

Therefore, hand hygiene is one of the most important aspects in the prevention and control of HAIs (Sosa, 2019): hand washing should be included in a wider hygiene program that involves developing and following standard hygiene protocols for patient care to avoid the transmission of microorganisms between patients, between the environment, and patients and health personnel (Patiño and Morales, 2013). For instance, the WHO has promoted hand washing through its multimodal hand hygiene improvement strategy and has shown that this can prevent up to 50% of avoidable HAI at a very favorable cost to benefit (MINSAs, 2016). To target these microbes, antiseptics are recommended for hand hygiene: alcohol gel (AG) and chlorhexidine foam (CF) have been recommended (MINSAs, 2016).

AG preparations containing 60 to 95% ethanol in water are most frequently used and have broad activity against vegetative bacteria, viruses, and fungi, but are not sporicidal (Gold, et al., 2022). This includes both Gram-positive and negative bacteria, as well as vancomycin-resistant *Enterococcus* or methicillin-resistant *Staphylococcus*, and *Mycobacterium tuberculosis* (Cano-González et al., 2020). Alcohol solutions act by denaturing proteins or through membrane damage.

Chlorhexidine is a light-sensitive biguanide that is effective against Gram-positive and Gram-negative bacteria, yeasts, and enveloped viruses. It is not sporicidal and has little effect against *Mycobacterium tuberculosis*. Chlorhexidine achieves its effect within 20 seconds: at low concentrations, it alters the osmotic permeability of the cell membrane, while at high concentrations, it precipitates proteins and nucleic acids. For hand hygiene, a 2 to 4% CF or a combination of alcohol-chlorhexidine foam is commonly used.

The Peruvian Ministry of Health (MINSAs-Peru) established a technical guide that includes procedures for both antiseptics (MINSAs 2016; Sosa 2019), which are based on World Health Organization recommendations (WHO, 2009b). Therefore, it is reasonable to

question which antiseptic is best given a set of conditions, but scarce or conflicting data exist, especially for real-world scenarios. Therefore, the efficacy of AG and CF for hand hygiene of nurses in two Peruvian hospitals was evaluated.

MATERIAL AND METHODS

Study participants

Participants were recruited among nurses and nursing technicians (NNT) employed at Belén Hospital (HB) and Trujillo Regional Teaching Hospital (HR), both located in Trujillo, Peru, between April and October 2020. The NNT belonged to the surgical center, the emergency department, or the adult or neonatal ICU. The study was approved by the Institutional Research Ethics Committee of the School of Medicine of the National University of Trujillo, approval code 212-2020-UNT-FM-C.E. Written informed consent was obtained from the participants and the recommendations of the Belmont report were followed. The study population consisted of 123 NNT at both hospitals. Participants were excluded if their hands were visibly dirty at the time of the test or if they had allergies to the antiseptics.

A pilot study comprised of 11 nurses was conducted to estimate the expected mean and standard deviation of microbial reduction, which was then used to estimate the sample size required for a 95% significance level and 90% test power (Norman and Streiner, 1998). The results of this pilot study suggested a sample size of 48 for each of the two treatment groups.

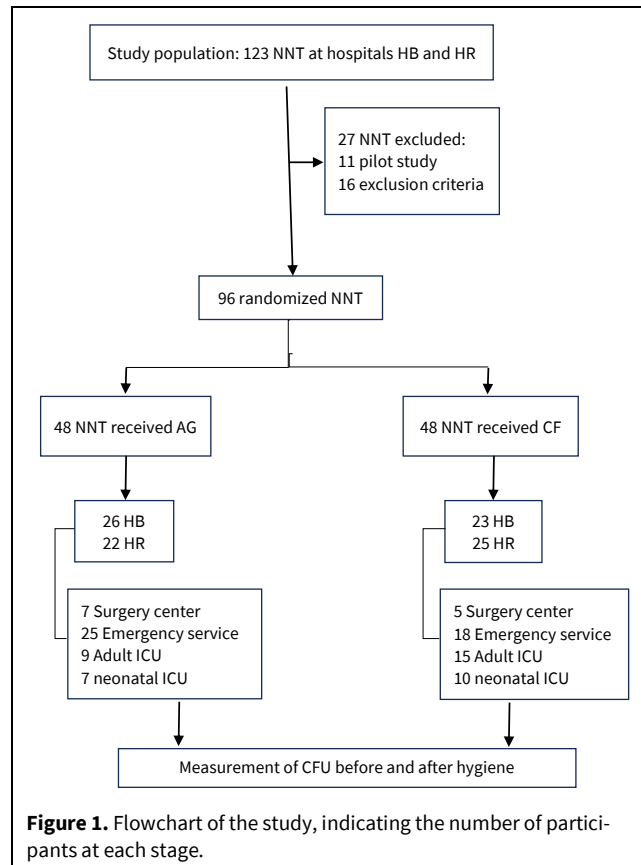
Study design

Participants were randomly assigned in two groups of 48 to use AG or CF, with proportional assignment of the hospital, service, and occupation (professional or technical) of the nursing staff. Each treatment group was divided into these subgroups for disaggregated analysis. The assignment of the group and the number of participants at each stage are listed in Fig. 1.

Procedure

The authors first trained the study participants in the WHO-recommended clinical handwashing technique (WHO, 2009b) and confirmed that the technique was followed where appropriate. The researchers later visited the participants at work to take two microbial samples from the hands of each participant.

The first sample was taken before hand hygiene by immersion of both hands in 50 mL of 1% sterile



peptone water contained in a self-closing transparent polyethylene bag sterilized with ethylene oxide.

The second sample was taken using the same protocol, but after hand hygiene following the WHO-recommended technique using one of two randomly assigned antiseptics: 70% AG (100 mL contains 73 mL 96% ethanol, 0.2 g carbomer, 5 mL aloe vera, 0.5 mL lemon essence, 0.1 mL triethanolamine, balance purified water; purchased from Biogel Laboratories, Peru) or 2% CF (100 mL contains 2 g chlorhexidine gluconate, balance excipient; from Laboratorios D. A. Carrión S.A.C., Peru).

The samples were kept in a cooler containing Blue Ice refrigerant blocks to maintain a temperature between 4 and 8°C to avoid an increase in CFU/mL before the culture of the samples. The temperature was confirmed using a thermometer (Boeco, Germany, range -50 to +50°C), which remained in the cooler while the samples were inside. Less than two hours after sample collection, the samples were transported to and processed at the Microbiology Laboratory of the School of Medicine of the National University of Trujillo.

Trypticase soy agar plates were seeded with the peptone broth sample (0.01 mL) using a calibrated loop and surface seeding; inoculated plates were incubated at 37°C for 24 hours. After incubation, the

resulting colonies were counted and multiplied by 100 to determine CFU/mL for each sample. The personnel who cultured the samples and reported the results were unaware of the antiseptic used by each participant (single-blind).

Efficacy for each antiseptic was measured by taking the CFU/mL measurement difference before and after hygiene for each patient. In the analysis, the hospital (HB, HR), type of hospital service (surgical center, emergency service, adult in intensive care unit [ICU], neonatal ICU), and the occupation of the nursing staff (professional nurses, nurse technicians) were considered.

Statistical analysis

The CFU/mL measurements before and after the treatment were submitted to the Kolmogorov-Smirnov normality test. A normal distribution was not observed, so a nonparametric analysis was completed. Following the first strategy, the nonparametric Wilcoxon test was used to evaluate the reduction of CFU/mL before and after treatment as correlated variables, followed by the Mann-Whitney U test to compare the reductions between AG and CF treatments (Norman and Streiner, 1998). Furthermore, a multivariate analysis of principal components (PCA) that reduces dimensions was applied to reorder the

categories and treatments of AG and CF in two dimensions (Jolliffe and Cadima, 2016). The level of significance was set at $p < 0.05$. SPSS version 26 software was used to complete the statistical analysis.

RESULTS

Participant information

The results of the pilot study (reduction of 8547 ± 7985 and 3260 ± 2131 CFU/mL for AG and CF, respectively) suggested a minimum of 96 participants (48 per treatment group) to reach the desired level of confidence. According to hospital records, the study population was 123 persons (75 nurses and 48 nurse technicians); 96 completed the study.

Table 1 reports the distribution of the participants between hospital, occupation, and service. The homogeneity test did not find significant differences among the distributions for both treatments.

CFU/mL reduction

Table 2 compares the CFU/mL before and after treatment measured for AG and CF in aggregate or by hospital, occupation, and service. The "treatment" columns report the results of the Wilcoxon test before and after hand antisepsis using AG and CF. Both treatments significantly reduced the measured

CFU/mL, regardless of how the participants were grouped. The "comparison" columns compare treatment efficacy using the Mann-Whitney U test. Only participants in the surgical center had a statistically significant greater reduction in CFU/mL in favor of CF. All other groupings of participants did not show a statistically significant difference between treatments.

Multivariate analysis of dimension reduction

The multiple matching program stopped after 65 iterations, after which it reached the proof of convergence value. The reduction of variables to two dimensions is described (Fig. 2):

Dimension 1 (abscissa axis) covers an inertia load of 0.431 and explains 43.1% of the total variance. The categories of the service variable and those of the profession variable are in this dimension.

Dimension 2 (ordinate axis) covers an inertia load of 0.350 and explains 35.0% of the total variance. The categories of the hospital variable are in this dimension.

The categories form a cluster whose accumulation point is the coordinate (0,0); the closest to the center of the biplot are the two antiseptics; on the other hand, the exclusion of the Surgical Center category was observed.

Table 1. Study participants divided between hospital, occupation, and service as well as treatment (AG or CF).

Participant group	Treatment				Homogeneity test	
	AG (n=48)		CF (n=48)		X ²	P
	N°	%	N°	%		
Hospital					0.375	0.540
HB	26	54.2	23	47.9		
HR	22	45.8	25	52.1		
Occupation					0.044	0.834
Professional nurses	29	60.4	30	62.5		
Nurse technicians	19	39.6	18	37.5		
Service					3.502	0.320
Surgical center	7	14.6	5	10.4		
Emergency	25	52.1	18	37.5		
Adult ICU	9	18.8	15	31.3		
Neonatal ICU	7	14.6	10	20.8		

AG: 70% alcohol gel; CF: 2% chlorhexidine foam; X²: Chi squared; P= p-value. HB: Belén Hospital; HR: Regional Hospital.

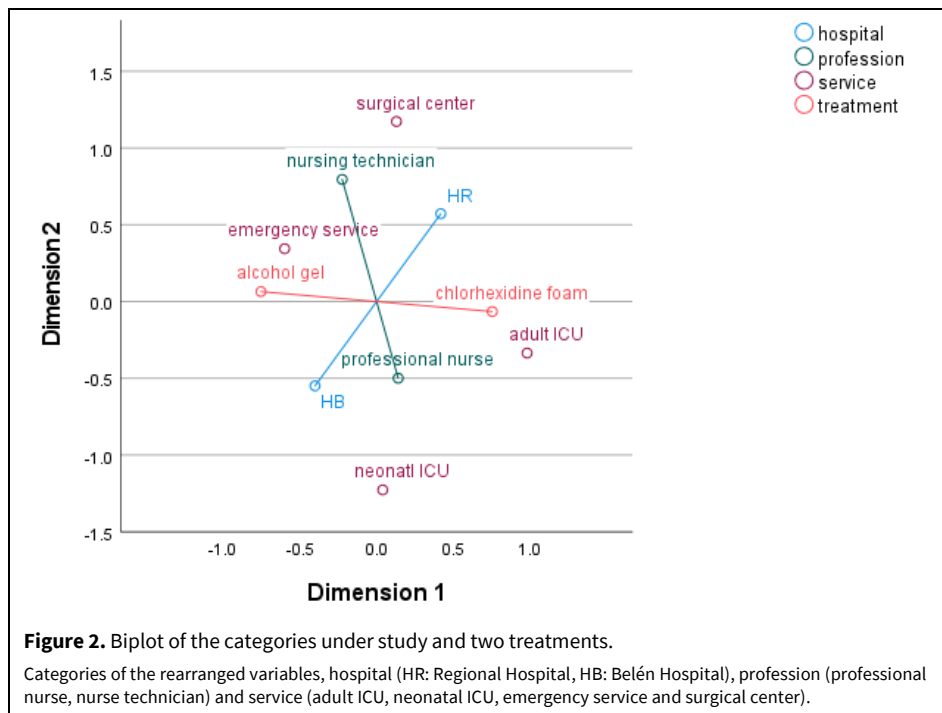
Table 2. Descriptive statistics of CFU/mL measurements before and after antiseptis with AG or CF for all participants (Total) and for different groupings of participants.

Participant group	Treatment				Mann Whitney U comparison between treatments (Z, p)	
	AG (n = 48)		CF (n = 48)			
	CFU/mL before	CFU/mL after	CFU/mL before	CFU/mL after		
Total					-1.495	0.135
Mean	9.108	1.742	6.050	529		
Standard deviation	9.794	3.099	7.561	801		
% Reduction		80.9		91.3		
Wilcoxon (before-after)	Z = -6.031	p=0.000	Z = -6.032	p=0.000		
HB	(n = 26)		(n = 23)		-1.252	0.210
Mean	10.754	1.165	1.961	543		
Standard deviation	10.507	1.208	8.853	698		
% Reduction		89.2		72.3		
Wilcoxon (before-after)	Z = -4.458	p=0.000	Z = -4.198	p=0.000		
HR	(n = 22)		(n = 25)		-0.821	0.412
Mean	7.164	2.424	4.292	516		
Standard deviation	8.713	4.342	5.778	899		
% Reduction		66.2		87.9		
Wilcoxon (before-after)	Z = -4.107	p=0.000	Z = -4.374	p=0.000		
Professional nurses	(n = 29)		(n = 30)		-1.850	0.064
Mean	9.672	2.125	5.363	510		
Standard deviation	9.996	3.852	7.488	905		
% Reduction		78.0		90.5		
Wilcoxon (before-after)	Z = -4.704	p=0.000	Z = -4.873	p=0.000		
Nurse technicians	(n = 19)		(n = 18)		-0.01	1.000
Mean	8.247	1.158	7.194	561		
Standard deviation	9.681	1.182	7.757	611		
% Reduction		86.0		92.2		
Wilcoxon (before-after)	Z = -3.824	p=0.000	Z = -3.724	p=0.000		
Surgical center	(n = 7)		(n = 5)		-2.355	0.019
Mean	2.157	371	11.240	1.360		
Standard deviation	752	298	7.757	684		
% Reduction		82.8		87.9		
Wilcoxon (before-after)	Z = -2.366	p=0.018	Z = -2.023	p=0.043		
Emergency service	(n = 25)		(n = 18)		-1.712	0.087
Mean	10.328	1.908	5.567	511		
Standard deviation	10.600	3.767	7.849	940		
% Reduction		81.5		90.8		
Wilcoxon (before-after)	Z = -4.373	p=0.000	Z = -3.724	p=0.000		

Table 2. Descriptive statistics of CFU/mL measurements before and after antiseptics with AG or CF for all participants (Total) and for different groupings of participants (continued...)

Participant group	Treatment				Mann Whitney U comparison between treatments (Z, p)	
	AG (n = 48)		CF (n = 48)			
	CFU/mL before	CFU/mL after	CFU/mL before	CFU/mL after		
Adult ICU	(n = 9)		(n = 15)		-1.163	0.263
Mean	13.178	2.924	7.460	560		
Standard deviation	9.964	4.838	8.652	745		
% Reduction		77.8		92.5		
Wilcoxon (before-after)	Z = -2.666	p=0.000	Z = -3.408	p=0.000		
Neonatal ICU	(n = 7)		(n = 10)		-0.781	0.475
Mean	6.471	1.000	2.210	100		
Standard deviation	8.561	1.559	1.958	125		
% Reduction		84.5		95.5		
Wilcoxon (before-after)	Z = -2.366	p=0.018	Z = -2.803	p=0.005		

AG: 70% alcohol gel; CF: 2% chlorhexidine foam; CFU: colony-forming unit; Z: Z-test; p: p-value.



DISCUSSION

Hand antiseptics of healthcare personnel is a well-established first-line strategy to decrease the spread of pathogens in the healthcare setting. Proper antiseptics requires the correct technique and the appropriate antiseptic.

AG and CF are antiseptics recommended by the WHO and are used in health establishments in Peru (Cano-González et al., 2020; Diomedi et al., 2017;

MINSA, 2016; WHO, 2009a). These antiseptics achieve bactericidal, fungicidal, and virucidal activity when the alcohol base is greater than 60%, but is not sporicidal. The efficacy of AG can be influenced by contact time, application technique and excipients, such as glycerin (Saha et al., 2021; Tarka et al., 2019). On the other hand, chlorhexidine is an alcohol-free antiseptic derived from chlorine (Jing et al., 2020; Villa and Russo, 2021) that has a similar antiseptics profile. Although CF is available in concentrations between 0.5

and 4%, a 2 or 4% foam is used in Peru for hand antiseptics (IREN, 2012; WHO, 2009b).

In this work, the efficacy of AG and CF in hand antiseptics of health personnel from the emergency services, surgical center, adult ICU, and neonatal ICU of two hospitals in the La Libertad region of Peru were compared, providing a dataset obtained under real working conditions. Both treatment groups were found to not differ significantly according to the homogeneity test, which allows a comparison of efficacy between antiseptics and between different groupings of participants.

To make this study applicable to a real hospital setting, samples were taken from nursing staff present at their workstations as long as there was no visible hand contamination. Samples were collected both before and after hand antiseptics by dipping hands in a bag containing sterile 1% peptone water. This method is inexpensive, uses common components, and allows testing of all surfaces of both hands. Other methods, such as glove juice, hand swab, and impression of palms and finger pads on agar plates (Diomedi et al., 2017; Girou et al., 2002; Leal et al., 2015) do not have this combination of advantages.

Both AG and CF were observed to cause a statistically significant decrease ($p < 0.05$) in CFU/mL for all tested groups, with a large magnitude (Table 2). This indicates that both antiseptics are efficient at reducing CFU/mL. However, no statistically significant differences were found ($p > 0.05$) when the antiseptics were compared, except for the Surgical Center participants, where CF reduced CFU/mL more than AG ($p < 0.05$) (Table 2).

The analysis was complemented with the multivariate method of the main components, reducing the variables to two dimensions (Fig. 2). It was observed that the location of the AG and CF treatments coincide in this rearrangement; this explains the absence of statistical difference between them, that is, the effect of the two interventions would be at the same level of efficacy. Additionally, as seen in Table 2, this effect has a similar pattern in the disaggregated analysis, with the sole exception of the surgical center, which is located discriminated from the set of categories centered on the origin of the plot (Fig. 2). This makes it possible to reinforce the nonparametric statistical analysis carried out.

Other investigations have been conducted comparing different antiseptics, such as AG, CF, chlorhexidine-alcohol mixtures, and povidone-iodine, with varying degrees of agreement. For example, Diomedi et al. (2017) compared different soaps and antiseptics using the hand rub culture method and found that alcoholic chlorhexidine gluconate was the most effective,

but its efficacy depended on concentration. Using the sterile bag method, they found that the reduction of bacterial load of alcoholic chlorhexidine was superior to both isopropanol and 4% chlorhexidine. In this context, Arbogast et al. (2019) analyzed the efficacy of chlorhexidine-based soaps in a clinical trial among US health personnel and found that chlorhexidine formulations with three different concentrations complied with FDA criteria for antimicrobial efficacy. This contrasts with other studies, including this one, that found no difference between AG and 4% chlorhexidine antiseptics using swabs or fingertip and palm impressions to measure bacterial load (De La Cruz et al., 2013; Girou et al., 2002; Morejón and Notario, 2020). Furthermore, a clinical trial did not find significant differences between AG and CF; both protocols met bacterial reduction criteria (Edwards et al., 2017). Also, these antiseptics have been shown to be superior to povidone-iodine in two separate studies (Ho et al., 2019; Tsai et al., 2017). Given the variety of methods used to measure hand antiseptics and the multiple results between studies, it is likely that alcohol and chlorhexidine hand rubs are both effective hand antiseptics when directly compared. Therefore, factors play a larger role in hand antiseptics than the selection of antiseptic.

Some confounding variables in this study include the measurement method, the antiseptic formulation, and the hand hygiene technique. In this study, surgical center nurses had more experience using CF, as it is commonly used in surgery, which could explain the statistically significant difference (Table 2). When studies using different antiseptics measurements are compared (Arbogast et al., 2019; Diomedi et al., 2017; Girou et al., 2002; Morejón and Notario, 2020), the results are different. Additionally, the gelling agent and excipients in alcohol hand rubs, such as carbomer, hydroxypropylmethylcellulose, and hydroxyethylcellulose, influence the antiseptic efficacy of the final preparation (D'Angelo et al., 2022). Finally, although participants were trained in the WHO-approved hand hygiene technique, the variability of hand antiseptics techniques among study participants may explain the variability in results. This may be addressed in larger studies, where the confounders may be better controlled. Furthermore, this type of study can be carried out among different health professionals who frequently use antiseptics in the clinic.

Despite these disadvantages, it is reasonable to state that AG and CF are effective clinical hand antiseptics when used properly. This information will likely be useful to improve the selection criteria for the purchase and clinical use of hand antiseptics. Indeed, in Peru, AG and CF are used almost interchangeably.

CONCLUSION

This study shows that AG is as effective as CF in reducing the hand microbiota of nursing staff in two Peruvian public hospitals under real working conditions. In the global analysis, a CFU/mL reduction of 80.9% and 91.3% was achieved for AG and CF, respectively, but the difference in efficacy was not statistically significant. If the analysis is disaggregated by workplace category, the same tendency was observed, with CF showing a slight superiority, but without statistical significance ($p > 0.05$), except for the surgical center, where the difference was significant. Therefore, AG and CF should be considered equally effective antiseptics, and other factors such as cost, availability, and staff experience should be considered when choosing the appropriate antiseptic.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Contribution	Gil-Peña DR	Ayala-Ravelo MS	Neciosup-Obando JE	Campos-Florián JV	Huamán-Saavedra JJ
Concepts or ideas	x	x			x
Design	x	x			x
Definition of intellectual content	x	x	x	x	x
Literature search	x	x	x	x	x
Clinical trial	x	x			x
Experimental studies	x	x			x
Data acquisition	x	x			x
Data analysis	x	x	x	x	x
Statistical analysis			x		
Manuscript preparation	x	x		x	x
Manuscript editing				x	
Manuscript review	x	x	x	x	x

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