

## Surgical hand antisepsis: experimental study

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**Purpose:** Nosocomial infections account for one of the most serious complications in hospitalized patients around the world. Surgical site infections have significant economic implications, and surgical antisepsis plays an important role in such processes.

**Methods:** With prior approval by the Institutional Review Board and informed consent, 10 volunteers were randomly assigned to 3 protocols on hand antisepsis: protocol A (chloroxylenol 3%), protocol B (benzalkonium chloride at 1%), and protocol C (ethyl alcohol 61%, 1% chlorhexidine gluconate). Smears from both hands were cultured after each hand protocol (t0) and at the end of suturing (t1). Colony forming units were counted (CFUs on blood agar dishes) with digital counting software (Open CFU). Friedman test was used to compare the mean values among the groups, and a Bonferroni correction was made to determine the dissimilar group, with a  $P = 0.015$ .

**Results:** At t0 for protocol A the CFU count was  $82.8 \pm 1.3$ ; protocol B was  $9.7 \pm 30$ ; protocol C was  $0.1 \pm 0.3$  ( $P < 0.001$ ). At t1 for protocol A the CFU was  $80.7 \pm 89.4$ ; protocol B was  $7.5 \pm 32$ ; protocol C was  $0.0 \pm 0.0$  ( $P < 0.001$ ). No adverse events were present among the subjects.

**Conclusion:** Ethyl alcohol at 61% with 1% chlorhexidine gluconate showed higher efficacy than the traditional washing antiseptics.

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**Key Words:** Infection control, Antisepsis

### INTRODUCTION

Nosocomial infections represent one of the major sources of morbidity and mortality in hospitalized patients around the world. Out of those infections, the most common one is the surgical site infection (SSI) [1]. According to the World Health Organization, in developing countries, the risk of infection related to health care practices is from 2 to 20 times higher than that in developed countries [2]. The unnoticed transmission of bacteria to patients' wounds during surgery may cause SSI. SSIs are one of the most common causes of health-care related infections for patients who undergo surgery [3].

The bacteria that cause SSI come from different sources in the operating room, including hands and surgical equipment. All the team members use sterile gloves to prevent spreading

bacteria to the patient and vice versa. However, gloves may get punctured during surgery, thus it is important to have the hands germ-free. This can be achieved through surgical hand antisepsis right before wearing the gloves for the surgical procedure.

Simple hand washing, removes most of the transient skin bacteria, while hand antisepsis also inhibits the growth of resident bacteria, thus reducing the risk of SSI [3-8]. The characteristics of an ideal antiseptic would be: immediate action, persistent (lasting several hours), cumulative activity (repeated exposure provides enhanced inhibition of the bacterial growth), broad spectrum of activity, and safe use by the operating room staff [9,10].

There are several strategies involved in hand surgical antisepsis: prior washing, washing technique, use of sponges,

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brushes, nail cleaning spatulas, duration of the antiseptics, and the antiseptic agent used.

Different terms are used to describe hand surgical antiseptics. Traditional antiseptics involve a brush, an antiseptic agent, and running water. Water-free antiseptics are those that involve alcohol-containing solutions.

The Association for Perioperative Practice (AfPP) recommends washing the hands before applying antiseptics. Washing should consist of using soap or an antimicrobial substance under running water (AfPP 2011). The purpose of hand washing is to remove dust and major gross contaminants. After that, the recommendation is to use plastic spatulas to clean the nails under running water. Finally, to perform either a traditional or water-free antiseptics [11].

Therefore, surgical hand antiseptics are one of the major factors in reducing the transmission of bacteria [12]. There are not many studies on surgical hand washing and its effect in the reduction of bacterial colonization [13].

## Antiseptic agents

### *Alcohols*

The most widely used are ethanol, isopropanol and n-propanol. They are effective against gram positive and gram-negative bacteria, mycobacterium tuberculosis and many viruses, and fungi [14].

### *Iodophors*

They are usually associated to polyvinylpyrrolidone to extend its duration, are effective against gram-positive, gram-negative bacteria, mycobacterium tuberculosis, and viruses [15].

### *Chlorhexidine*

It is a biguanide effective against gram-positive, gram-negative bacteria, lipophilic viruses and yeasts, nonsporidicidal. It has a long-lasting action because it attaches to the horny layer of the skin [16].

### *Quaternary ammonium compounds*

The benzalkonium alkyl-chloride is the most used for hand antiseptics. It is bacteriostatic, fungistatic, and microbicidal at high concentrations. It has a higher activity against gram-positive bacteria than gram-negative and its activity is weak against mycobacterium and fungi. It has very good activity against lipophilic viruses. The U.S. Food and Drug Administration (FDA) has not fully approved its efficacy and safety as an antiseptic [8].

### *Chloroxylenol*

It is a phenolic compound with a halogenated substitute. Its action is NOT as quick as that of the chlorhexidine or iodophors and it exhibits a short residual activity. The FDA has not fully

approved its efficacy and safety as an antiseptic [17].

Today, there is a large amount of antiseptics with various active ingredients whose properties have not been analyzed in our setting that could be very useful for hand washing and thus reduce the transmission rate of infections [18-20].

In this work, 3 surgical hand antiseptics methods are compared in order to measure their effectiveness in reducing the bacterial colony forming units, as well as to evaluate if those antiseptics provide a long-lasting antibacterial protection effect.

## METHODS

### Study design

After approval by the research committee, 10 volunteers were recruited for this experimental study. A simple randomization with no repetitions was carried out for 3 antiseptics. The experiment was conducted during a suturing workshop in an experimental laboratory fitted with the regular equipment for surgeries and hand washing stations. The workshop consisted of 3 different suturing sessions (1 hour each), where subjects were subjected to a supervised surgical hand washing protocol before starting their suturing practice. Samples were taken from the finger pulps before (t0) and after (t1) each suture.

The inclusion criteria were: medical students with prior experience in hand washing in a surgical setting. Subjects were not allowed to have history of infections in upper limbs or recent trauma in fingers or hands. All subjects were informed about the risks and benefits of the study and informed consent was requested from them to participate in the study.

Upon signing the informed consent, an informative video was shown to them explaining how to do the hand washing according to the protocols involved. The video was played again at the moment of conducting the experiment.

Before each surgical washing protocol, all participants spent 1-minute washing their hands with neutral soap and a sponge brush in order to eliminate the gross contaminants, and a checklist and a timer were used.

### Details of the hand washing protocol

The study participants were randomized to 3 hand antiseptics protocols 1 week apart:

Protocol A: 3 minutes of traditional washing with BD E-Z Scrub 116 (chloroxylenol 3%; Becton Dickinson, Sandy, UT, USA) followed by 1 hour of suturing practice.

Protocol B: 3 minutes of traditional washing with Antibenil (benzalkonium chloride at 1%; Farmaceuticos Altamirano, Mexico City, Mexico) followed by 1 hour of basic suturing practice.

Protocol C: 1 minute of antiseptics with Avagard (ethyl alcohol 61%, 1% chlorhexidine gluconate; 3M, St Paul, MN, USA) followed by 1 hour of basic suturing practice.

Finger pulps were gently smeared to get the culture on 2 blood agar dishes (one for each hand) for 5 seconds (t0). Right immediately, participants put the gloves on using a sterile technique and conducted their suturing practices (sutures, knots) as part of the workshop activities. One hour later, their gloves were removed with a sterile technique and smears were taken from both hands to culture them on blood agar dishes (t1).

The blood agar dishes had a commercial preparation (DIBICO; DIBICO SA DE CV, Cuatitlan Izcalli, Mexico) sensitive to *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*.

Five control tests without hand washing were conducted by placing the finger pulps on 2 agar dishes respectively and making sure the culture was done properly.

Each experiment session was one full week apart to prevent residual factors from some of the antiseptics and thus altering the results.

The investigation coordinators (ID, CM) were present at

each session (initial hand washing, surgical antisepsis, drying, gloving, suture practicing, and glove removal) to guarantee the appropriate technique and compliance with sterility procedures.

The agar dishes were delivered to the Microbiology Department and were immediately placed in incubators at 35°C ± 2°C at a pressure of 5% of CO<sub>2</sub>. After a 48-hour incubation, the blood agar dishes were taken out of the incubator and the CFUs were counted manually by a technician blind to the protocols, and the dishes were photographed for digital processing (Fig. 1). With an open source software, Open CFU (Quentin Gleissman, Sheffield, UK), the measurements were verified and the interclass correlation coefficient, between the observer and the software, was estimated.

### Data analysis

The CFU count distribution was expressed as a mean and as a standard deviation. The before-after comparison for paired data was made with the Wilcoxon signed-rank test. The comparison among groups was performed with the Friedman test and a Bonferroni adjustment was made to delimit the differences among the groups with a P < 0.015, which was considered significant. The entire statistical analysis was conducted by using IBM SPSS Statistics ver. 24.0 (IBM Co., Armonk, NY, USA).

## RESULTS

### Demographic description

Ten subjects were included with a mean age of 21.1 ± 1.3 years, 50% of the participants were women. No adverse events were found in any subject.

### Antiseptic efficacy

The results of the comparison of the antiseptic efficacy in each participant, and in each hand, are shown on Table 1.

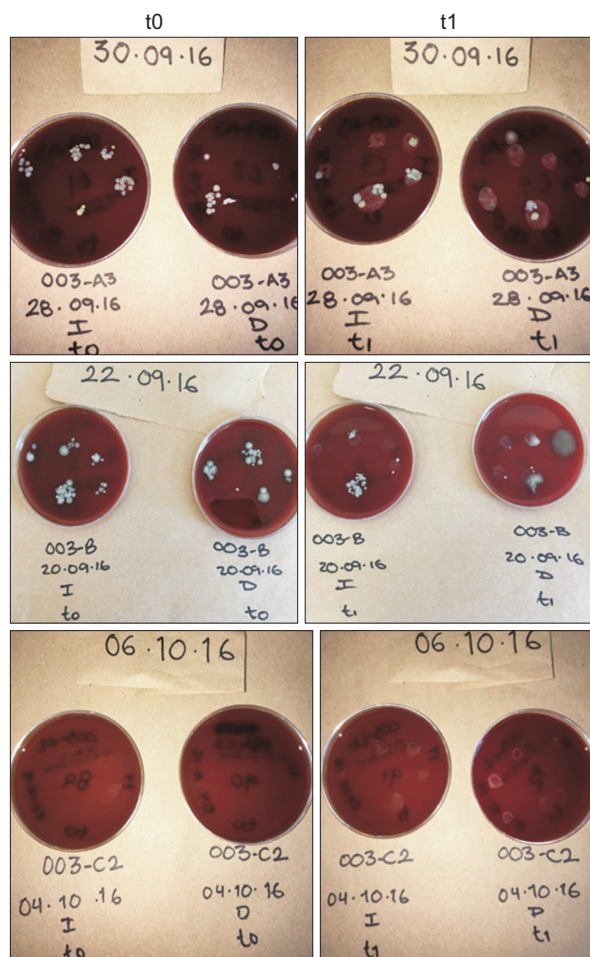
At the initial time (t0) with protocol A the CFU count was 82.8 ± 1.3; with protocol B, 9.7 ± 30; with protocol C, 0.1 ± 0.3 (P < 0.001). At the end of the suturing practice session, the CFU count measurements were (t1) for protocol A, 80.7 ± 89.4; protocol B, 7.5 ± 32; protocol C, 0.0 ± 0.0 (P < 0.001).

In order to validate our measurements, the CFU manual measurement was compared to that of the Open CFU software and the interclass correlation coefficient was 0.99.

Expressed as ratios, in protocol A there was bacterial growth in 100% of the agar dishes, in protocol B at the initial time, 40% of subjects had CFU and at the final time, 30%. In protocol C, there was only one CFU in 2 subjects at the initial time and none at the final time.

### Before-after comparisons

We did not find any reduction in the amount of CFUs accounting for a statistically significant difference in any antiseptic over



**Fig. 1.** Colony forming units on blood agar dishes. A, chloroxyleneol; B, benzalkonium chloride; C, alcohol 61% y chlorhexidine at 1%. D, right, I, left. Before the practice (t0) after the practice (t1).

**Table 1.** Colony forming units with different antiseptics

Subject	Hand	Protocol A		Protocol B		Protocol C	
		t0	t1	t0	t1	t0	t1
1	Right	9	3	0	0	1	0
	Left	9	3	0	0	0	0
2	Right	55	25	1	0	0	0
	Left	25	55	3	0	0	0
3	Right	20	3	125	4	0	0
	Left	40	20	60	145	0	0
4	Right	75	71	1	0	0	0
	Left	75	96	0	0	0	0
5	Right	65	40	1	0	0	0
	Left	25	25	0	1	0	0
6	Right	121	75	0	0	0	0
	Left	140	155	0	0	0	0
7	Right	50	285	1	1	0	0
	Left	140	155	0	0	0	0
8	Right	27	47	0	0	0	0
	Left	10	55	0	0	0	0
9	Right	18	9	0	0	1	0
	Left	15	12	0	0	0	0
10	Right	421	184	1	0	0	0
	Left	317	296	1	0	0	0

Colony-forming unit count from the 5 fingers of each hand immediately after surgical washing (t0) and after the suturing practices (t1) among subjects of the study.

A, chloroxylenol; B, benzalkonium chloride; C, alcohol 61% y chlorhexidine at 1%.

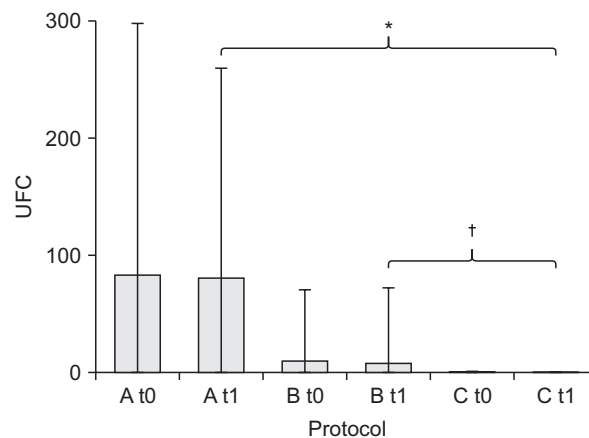
time (Fig. 2). The most efficacious antiseptic for CFU prevention was the one in protocol C, and there was a statistically significance both at the initial and final periods ( $P < 0.001$ ).

## DISCUSSION

Hand antisepsis among staff dealing with surgeries is crucial in preventing SSIs. In this experimental study, we compared 3 antiseptics commonly used in our region. We assigned the order of the surgical antisepsis protocols randomly and we measured them before and after a suturing practice session. We found that the combination of alcohol at 61% and chlorhexidine for 1 minute, is more efficacious than other antiseptics available.

Other authors have reported this finding. In a study from Singapore, where povidone iodine was compared against chlorhexidine and alcohol in 10 subjects, the efficacy to prevent CFU growth was higher in the latter group. Interestingly, povidone iodine enhanced the antiseptic protection over time and eliminated the CFU development one hour after the hand preparation [18].

In a study done in Taiwan by Tsai et al. [19], in a clinical trial with 236 patients, povidone iodine was compared to soap with



**Fig. 2.** Bar graph comparing the amount of CFUs with 3 antiseptics: A, chloroxylenol; B, benzalkonium chloride; C, alcohol 61% y chlorhexidine at 1%. Note that in groups B and C there was a reduction in CFUs, however almost null growth was observed in group C. Values are presented as mean  $\pm$  2 standard deviation. \*Wilcoxon signed test A vs. C t1,  $P < 0.001$ . †Wilcoxon signed test B vs. C t1,  $P = 0.027$ . t0, time zero; t1, time after practice.

chlorhexidine and ethyl alcohol with chlorhexidine gluconate. In this study they found that the amount of CFUs growth with soap and chlorhexidine and chlorhexidine/alcohol was more efficacious than povidone iodine.

Herruzo-Cabrera et al. [20], compared 4 alcohol solutions to regular washing products (chlorhexidine and povidone iodine) *in vitro* (pig skin) and *in vivo*. The *in vivo* test was performed on healthy individuals with a cross-over design, with 154 members of the surgical team, on whom the microbial flora was measured before and after antisepsis, and before and after surgery. In this study, the quantitative, semiquantitative, and qualitative results favored the solution with alcohol without brushing on volunteers and surgeons alike.

In an interesting study carried out by Pietsch [21], an alcohol-based antiseptic was compared to a water-based one and the degree of skin irritation, efficacy in reducing bacteria before and after surgery, were compared. Also, an alcohol-containing antiseptic was compared to alcohol-containing antimicrobial gels. Fewer adverse events were seen in subjects who used an alcohol-based antiseptic with lower rates of bacterial growth compared to the water-based solution and gels.

Hajipour et al. [22] described in a randomized clinical trial the comparison between chlorhexidine gluconate and an alcohol gel as disinfectants. In the first part, all surgeons followed the traditional antisepsis protocol by using chlorhexidine under running water with a brush for 5 minutes in the first case. After the first case, surgeons were divided into 2 groups: one with alcohol gel and another one with chlorhexidine gluconate. At the end of each procedure, a culture was taken from the fingertips of each surgeon. After 41 surgical procedures and 82



hand washings, 4 hands had contamination (8%) compared to 19 hands in the group that used alcohol gel (34%), and the result was statistically significant. Also, the CFU count was higher in the group that used alcohol gel compared to the group that used chlorhexidine.

Regarding the surgical washing time, Kappstein et al. [23] compared a reduced antisepsis time (3 and 2 minutes) to the 5-minute standard time. The protocols they used were: 3 minutes with alcohol disinfectant, and 2 minutes with chlorhexidine and alcohol at 70%. They found that the time-reduced protocols were equally effective as the traditional procedure and that 2,360 hours and 79,768 German marks could be saved or 3,540 hours and 119,652 German marks, respectively.

In this experiment, to eliminate the risk of contamination, we took great care to carefully apply the antiseptic method as described by each of the manufacturers. A research coordinator, was in charge of verifying the proper technique of hand antisepsis that was done after thoroughly washing both hands with a normal soap. After meticulous culturing, the petri boxes were photographed. Two independent observers quantified the colonies that grew, and computer software was explored to leave human error or bias out of the equation. We consider that the result in a single subject in B antiseptic, and all patients with A antiseptic, can reflect potential problems in a surgical setting. Although contamination remains as a possibility, our concern is that antiseptics may have suboptimal results, and/or bacterial resistance to antiseptics is present [24-26].

Our study has some limitations. It was performed at the surgical laboratory of our school and its applicability to the clinical setting is difficult. The sample size is small, but given the large differences found among the groups, it is statistically sufficient. Since this was a pilot study, we did not identify the specific bacteria in order to determine the potential species of

the infections in our region. Also, due to the lack of patients, we could not measure the extrinsic risk factors involved in the development of SSIs.

With our results, we observed that the alcohol-chlorhexidine combination significantly reduces CFU growth with a continuous elimination of bacteria lasting several hours after being applied. We believe that the relatively short application time could optimize the process and use of hospital resources, which could result in enhanced adherence by hospital staff. Traditional washing, on the contrary, requires vigorous and lengthy rubbing with brushes and antimicrobial solutions, which could cause significant damage to the skin, and is associated to higher costs of supplies such as brushes, sterile towels, running water at sinks.

In conclusion, according to our pilot study, rubbing the hands with an alcohol-based solution with an additional compound (chlorhexidine) showed superior efficacy in the reduction and maintenance of CFUs compared to benzalkonium chloride, and to chloroxlyenol brush.

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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