

■ Express reports

Comparison of residual antimicrobial activity of chlorhexidine-containing antiseptics: An express report

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Summary :

Background and objectives: It has been considered that chlorhexidine gluconate (CHG) possesses substantial persistent antimicrobial activity due to its residue on the skin. However, despite the availability of many formulations containing CHG, data supporting this notion are scarce. Therefore, the primary aim was to compare the residual antimicrobial activity of three major types of CHG-containing antiseptic formulations: 4% scrub detergent formulation, 0.5% aqueous solution and 0.5% ethanol formulation.

Materials and Methods: Six circular test sites were located on the left forearm and two circular test sites were located on right forearm of healthy subjects (n=3). One of the three major CHG antiseptic formulations was applied to the left forearm. The right forearm was the control (nothing was applied). Twenty minutes after CHG application, all test sites on the left and right forearm were inoculated with *Serratia marcescens*. Five minutes after inoculation, specimens from each test site were collected using cup scrub technique, and were then diluted and spread on the trypticase soy agar plates. Specimens from right forearm were collected in the same manner. Colony forming units (CFU) of each plate were counted after being cultured for 24 hours, and log reduction in bacterial count from base line (average CFU count of control test sites) was calculated for each test site.

Results: Average bacterial count of *S. marcescens* was 5.74 Log₁₀CFU from the test sites which were not treated with CHG formulation. Average bacterial count was 3.44 Log₁₀CFU for the test sites treated with 4% CHG scrub detergent formulation, and 2.71 Log₁₀CFU for the test sites treated with 0.5% CHG aqueous solution. No bacterial growth was observed in the test sites treated with 0.5% CHG ethanol solution. Average RF in bacterial count from base line was 1.32 for the test sites treated with 4% CHG scrub detergent formulation, 3.81 for the test sites treated with 0.5% aqueous solution and 4.53 for the test sites treated with 0.5% ethanol solution. Average RF value following 0.5% ethanol solution treatment was significantly greater than that of 0.5% CHG aqueous solution ($p<0.05$) and also that of 4% scrub formulation ($p<0.05$). Average RF value following 0.5% CHG aqueous solution treatment was significantly greater than that of 4% scrub formulation ($p<0.05$).

Discussion: Residual antimicrobial activities of 4% CHG scrub, 0.5% CHG aqueous and 0.5% CHG ethanol formulations were studied. Residual antimicrobial activity of 0.5% CHG aqueous solution was much greater than that of 4% CHG scrub formulation, and residual antimicrobial activity of 0.5% ethanol solution was significantly greater than that of 0.5% aqueous solution. These results suggest that it is not the amount of CHG contained in the formulation that affects the residual activity, but that combining CHG with ethanol does in a way that prolongs residual activity. Thus, we presume that the chemical features of ethanol, such as lipophilic and hydrophilic characters, play an important role, and allow CHG residue to easily reach deep into the stratum corneum, and be evenly adsorbed to the skin despite the natural presence of lipids.

Key words : chlorhexidine, residual antimicrobial activity, *Serratia marcescens*, cup scrub technique

Background

It is considered that chlorhexidine gluconate (CHG) exhibits substantial persistent antimicrobial

activity due to its residue on the skin^{1,2)}. Accordingly, CHG is well accepted and used in various clinical situations, such as skin preparation before surgical incision³⁻⁶⁾, skin preparation before catheter insertion^{7,8)}, bathing or shower before

surgical operation⁹⁻¹²), and surgical hand preparation^{13,14}). However there are opposing arguments that there are not enough evidences that support the notion of substantial persistent antimicrobial activity of CHG^{15, 16}).

On the other hand, there are many formulations which contain CHG, such as aqueous solutions, alcoholic solutions, and surgical scrub detergents. However, it seems that there are not enough data showing how strong the residual activity is or how long the residual activity will last for each formulation. Therefore, we aimed to clarify and to compare the residual antimicrobial activity of three major types of antiseptic formulations containing CHG.

1. Objectives

To compare the residual antimicrobial activity of 4% scrub detergent formulation, 0.5% aqueous solution and 0.5% ethanol formulation after application on the skin

2. Materials and Methods

Residual antimicrobial activity of a 4% scrub detergent formulation, a 0.5% aqueous solution and a 0.5% ethanol formulation were tested on the forearm of 3 healthy volunteers.

The CHG antiseptic formulations used in this study were as follows. The 4% CHG scrub detergent formulation was Hibiscrub[®] (Lot. 2092C). The 0.5% CHG aqueous solution was prepared by fortyfold dilution of 20 w/v% CHG aqueous solution (Hexizac[®] Disinfectant Solution 20%, Lot. 710687) using JP Sterile Purified Water. The 0.5% CHG ethanol solution was prepared by fortyfold dilution of 20 w/v% CHG aqueous solution (Hexizac[®] Disinfectant Solution 20%, Lot. 710687) using JP Ethanol for Disinfection (contained 76.9-81.4v/v% ethanol).

Circular test sites of 2.4cm were arranged on the anterior side of right and left forearms of each volunteer. Six test sites were located on the left

forearm and 2 test sites were located on the right forearm (Figure 1). Right and left forearms were scrubbed with non-antibacterial soap for 30 seconds and rinsed with running water for 30 seconds. Each forearm was then naturalized with 3 mL of Triton X-100. After rinsing with running water for 30 seconds, each forearm was wiped with sterilized paper towel and allowed to dry. The left forearm of each volunteer was treated with one of the three CHG formulations. The right forearm was the control and was left untreated. As for the subject assigned to be treated with 4% CHG scrub formulation, anterior side of left forearm was scrubbed for 60 seconds with 5 mL of the scrub formulation, and rinsed with running water for 20 seconds, and wiped with sterilized paper towel and allowed to dry. As for the subjects assigned to be treated with CHG aqueous solution or CHG ethanol solution, cotton swab was used for application of each formulation. Each CHG formulation was painted on the test sites of left forearm with a fully moistened cotton swab for 60 seconds. After each CHG formulation application, we waited for 20 minutes to allow drying of any volatile ingredients. Then, 15 μ L of *Serratia marcescens* (ATCC 14756) suspension, prepared appropriately for the evaluation of antimicrobial activities¹⁷), was inoculated on all 6 test sites of the left forearm. Five minutes after bacterial inoculation, specimens were collected by cup scrub technique from each test site using sterilized stainless cylinder with an inside diameter of 2.4 cm. Five milliliters of bacterial sampling solution contained CHG activity neutralizer (3% lecithin and 10% polysorbate 80) were poured into the stainless cylinder and scrubbed skin surface for 45 seconds with a sterilized plastic rod. Collected samples were diluted with 0.01 mol/L phosphate buffered saline and spread on the trypticase soy agar (TSA) plates immediately. The two test sites of right forearm (control) were also inoculated with *S. marcescens* suspension, and specimens were collected in the same manner as described above. Colony forming unit (CFU) of each plate was counted after a 24 hour culture at 30

degrees Celsius, and reduction factor (RF) in bacterial count from base line (average CFU count of control test sites) was calculated for each test site.

RF of each CHG formulation group was analyzed by the analysis of variance, and average RF value of each group was compared by analysis of Tukey's Honestly Significant Difference (HSD) test at the $p < 0.05$ level of significance. Statistical analysis was performed by JMP 5.0.1J software (SAS Institute Inc.).

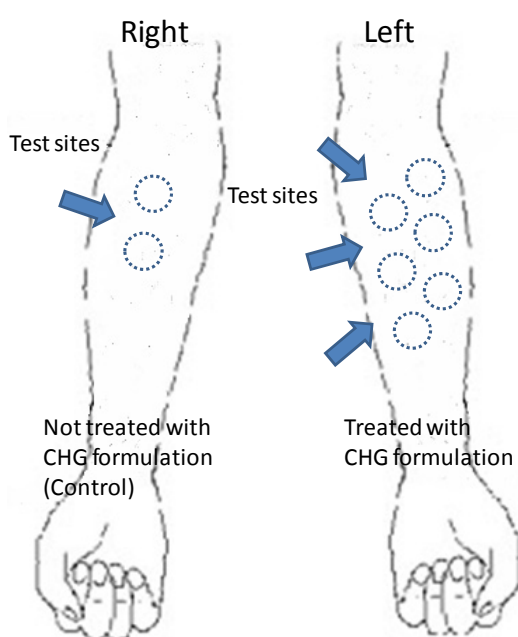


Figure 1. Test sites on anterior side of right and left forearms (2.4 cm diameter)

3. Results

From the test sites on the right forearm (not treated with CHG formulation), the average bacterial count of *S. marcescens* was 5.08 $\text{Log}_{10}\text{CFU}/\text{cm}^2$ ($n=6$). From the test sites on the left forearm treated with either 4% CHG scrub detergent formulation or 0.5% CHG aqueous solution formulation, average bacterial count was 2.78 $\text{Log}_{10}\text{CFU}/\text{cm}^2$ ($n=6$), and 2.05 $\text{Log}_{10}\text{CFU}/\text{cm}^2$ ($n=6$), respectively. No bacterial growth was observed in the TSA plates for the sample collected from the test sites treated with 0.5% CHG ethanol

solution (Table 1).

Table 1 Bacterial counts for the test sites treated with CHG formulations (Mean \pm SD, $n=6$)

	$\text{Log}_{10}\text{CFU}/\text{cm}^2$
Control	5.08 ± 0.98
4%CHG Scrub	2.78 ± 0.37
0.5%CHG Solution	2.05 ± 0.64
0.5%CHG Ethanol	<0.74 (not detected)

Average RF in bacterial count from base line was 1.32 for the test sites treated with 4% CHG scrub detergent formulation, 3.81 for the test sites treated with 0.5% aqueous solution and 4.53 for the test sites treated with 0.5% ethanol solution (Figure 2). Mean of RF value following 0.5% ethanol solution treatment was significantly greater than that of 0.5% CHG aqueous solution ($p < 0.05$) and 4% scrub formulation ($p < 0.05$). Also, mean of RF value following 0.5% CHG aqueous solution treatment was significantly greater than that of 4% scrub formulation ($p < 0.05$).

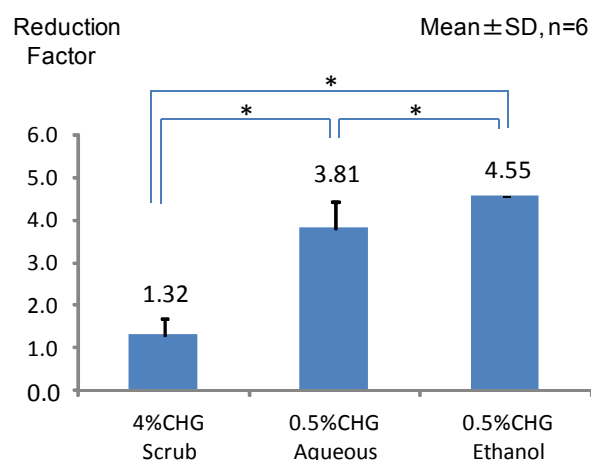


Figure 2 RFs for the test sites treated with CHG formulations
* $p < 0.05$ (Tukey's HSD test)

4. Discussion

Residual antimicrobial activity of 3 major types of antiseptic formulations containing CHG, such as a 4% scrub detergent formulation, a 0.5% aqueous solution formulation and a 0.5% ethanol solution were investigated on the forearm of healthy volunteers. In order to evaluate the residual activity of each CHG formulation, *S. marcescens*

suspension was inoculated 20 minutes after each CHG application. After 5 minutes, bacterial samples were collected and numbers of viable bacteria were counted.

The data obtained indicated that residual antimicrobial activity of 4% scrub formulation was markedly weaker than the 0.5% aqueous solution formulation and the 0.5% ethanol formulation. In addition, residual antimicrobial activity of 0.5% ethanol formulation was significantly greater than that of 0.5% aqueous solution.

These findings suggest that the amount or the state of residual CHG remaining on the skin after application of each CHG antiseptic formulations may differ, and that residual activity may not depend on the amount of CHG contained in the formulation. It appears that a 4% CHG scrub formulation may not be appropriate to provide sufficient residual antimicrobial activity although the concentration of CHG is highest.

Stahl JB *et al.* investigated and reported that residual antimicrobial activity of chlorhexidine on the skin was reduced after saline rinse or saline soak¹⁷⁾. Based on this, chlorhexidine residue may not be adsorbed deeply or bound tightly on the skin, and may relatively easily be removed from the skin surface. In addition, a scrub formulation contains many kinds of additional ingredients, such as surfactants, frothing agents, thickeners, emollients, and/or moisturizers etc. These additional ingredients, either alone or combined with each other, may negatively influence the adsorption of CHG residue on the skin surface.

A recent Cochrane Review with regard to the efficacy of preoperative bathing or showering on surgical site infection prevention referred to 7 clinical trials. The same 4% CHG detergent antiseptic formulation was used for bathing or showering in these 7 clinical trials. The review concluded that there was no clear evidence of benefit for preoperative showering or bathing with chlorhexidine to reduce surgical site infection¹⁹⁾. However, application method, such as dosage, timing of bathing/showering, number of multiple

applications etc., was poorly managed and different in each of the 7 clinical trials. It should be re-considered whether 4% scrub formulation is the best antiseptic and also what application method is recommendable to expect residual antimicrobial activity.

Interestingly, our results indicated that 0.5% CHG ethanol solution exhibited strongest residual antimicrobial activity, even stronger than 0.5% CHG aqueous solution. This suggests that the amount residual CHG compound or the state of residual CHG compound on the skin might be altered when applied in combination with ethanol. There are some reports which indicate stronger residual antimicrobial activity of CHG alcoholic solution compared with alcohol²⁰⁻²²⁾, or compared with aqueous CHG²⁰⁾.

However, the mechanism by which CHG residue is enhanced by ethanol remains unclear. Karpanen TJ *et al.* investigated the permeability into the skin of a CHG alcoholic solution and a CHG aqueous solution using an ex vivo human skin model, and concluded that CHG penetration was poor and limited following the application of either alcoholic or aqueous solutions²³⁾. On the other hand, Van der Merwe D *et al.* investigated the effects of ethanol on stratum corneum, and stated that ethanol/water mixture altered the stratum corneum through lipid extraction²⁴⁾. Thus we presume that the chemical features of ethanol, such as lipophilic and hydrophilic characters, play an important role, and allow CHG residue to easily reach deep into the stratum corneum, and be evenly adsorbed to the skin despite the natural presence of lipids.

Further study will be necessary to clarify the state and the mechanism responsible for enhancing skin CHG residue by ethanol. It is also necessary to investigate how long residual antimicrobial activity is maintained and how CHG residue on the skin decreases over time for each CHG-containing antiseptics.

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