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Comparative Study on the Antimicrobial Effect of 0.5% Chlorhexidine Gluconate and 70% Isopropyl Alcohol on the Normal Flora of Hands

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A gloved-hand wash method was used to compare the antimicrobial effect of chlorhexidine gluconate alcohol emollient hand wash (HIBISTAT) with that of 70% isopropyl alcohol on the normal flora of the hands (81 subjects) under conditions designed to mimic use by surgeons. Results of the immediate postwash effects on the bacterial counts for all 3 test days showed that chlorhexidine significantly reduced the normal flora of the hands. When compared with the base line bacterial counts, there was 85, 96, and 98% reduction with chlorhexidine treatment and 84, 93, and 90% reduction with alcohol treatment on days 1, 2, and 5, respectively. The difference between chlorhexidine and alcohol treatments was not statistically significant on days 1 and 2, but was significant on day 5 ($P < 0.01$). For delayed postwash bacterial counts (for persistent antimicrobial effects), the overall log means were 4.9943 and 5.4684 for chlorhexidine and alcohol treatments, respectively. The difference between the two treatments was significant ($P < 0.01$). After the chlorhexidine treatment, there was no significant growth of bacteria over a period of 6 h when compared with the base line bacterial counts.

Chlorhexidine has broad-spectrum antimicrobial activity against gram-positive and gram-negative bacteria (1, 6). Chlorhexidine exerts its antimicrobial effect by increasing the permeability of the cell membrane, with subsequent loss of cytoplasmic precipitation (2–4, 10).

This study investigates: (i) the immediate effect of 4% chlorhexidine gluconate alcohol emollient hand wash (HIBISTAT) in reducing the resident bacterial flora of the hands compared with 70% isopropyl alcohol; and (ii) the persistent effect of alcoholic chlorhexidine compared with 70% isopropyl alcohol alone in maintaining the reduction of normal microbial flora of the hands under conditions designed to mimic use by surgeons.

MATERIALS AND METHODS

Pretest period. During the pretest period, 14 days before bacterial sampling, the subjects avoided use of any medicated soaps, lotions, shampoos, and deodorants with antimicrobial activity. This period allowed the natural resident flora of the subjects' hands to stabilize.

Base line period. The base line period was 1 week after the 2-week pretest period. Bacterial counts were performed on days 1, 3, and 7 by the sampling technique discussed under "Glove fluid sampling procedure." This information provided evidence to assess the assumption that the right and left hands gave comparable counts. Both hands were sampled for the base line bacterial count. Subjects did not wash before the sampling procedure on the day of the experiment.

The base line procedure was: the hands, including two-thirds of the distance from the wrist to the elbow, were washed for 30 s with nonmedicated soap under warm running tap water at 35 to 40°C. Excess water was shaken from the hands, and the gloves were donned with the hands wet. After the hands were massaged, bacteria were cultured on appropriate culture media.

Experimental procedure. Eighty-four subjects were assigned at random to each treatment group (42 subjects in the chlorhexidine group and 42 in the alcohol group). Eighty-one subjects completed the study (41 in the chlorhexidine group and 40 in the alcohol group). Hands were treated with alcohol or chlorhexidine once before sampling on test days 1, 2, and 5, two additional times after sampling on test day 2, and three times on test days 3 and 4. Five milliliters of the assigned test preparation was vigorously rubbed over the hands and fingers and to 4 inches (10 cm) above the wrist line until dried. The sampling schedule for each treatment is given in Table 1.

Hands were sampled for bacteria on test days 1, 2, and 5 at the times indicated in the sampling schedule, following the methodology outlined below, with the right hand always being sampled immediately after treatment for immediate effect and the left hand randomized as described under sampling schedule for each treatment group for persistent effect.

Glove fluid sampling procedure. After the treat-

TABLE 1. *Sampling schedule for each treatment group using 0.5% chlorhexidine or 70% alcohol as hand wash*[a]

Days of sampling bacteria	No. of hands sampled at indicated hours after treatment						
	0	1	2	3	4	5	6
1	41[b] (40)[c]	7 (6)	6 (7)	7 (7)	7 (7)	7 (6)	7 (7)
2	41 (40)	7 (6)	6 (7)	7 (7)	7 (7)	7(6)	7 (7)
5	41 (40)	7 (6)	6 (7)	7 (7)	7 (7)	7 (6)	7 (7)

[a] On days 2 and 3 during the treatments, all subjects washed their hands three times with chlorhexidine or alcohol at 2- to 3-h intervals. Bacteria were removed at 0 h by gloved-hand wash method from both hands on days 1, 3, and 7 for base line studies before the treatments.

[b] Washed with chlorhexidine.

[c] Washed with alcohol.

ment, surgical gloves were donned, and 75 ml of sterile 0.1% Triton X-100 in phosphate-buffered (pH 7.8) solution was immediately added to the glove on the right hand (immediate postwash effect). The fluid under the glove was massaged by rubbing the hand thoroughly for 60 s. The gloves were removed and shaken gently to disperse bacteria evenly in the fluid. One milliliter of the glove fluid was removed and serially diluted. The glove on the left hand remained for the duration of the assigned testing time (1, 2, 3, 4, 5, or 6 h), with six to seven subjects being sampled at each interval (Table 1). The left hand was sampled in the same manner as the right hand. On day 2, a single wash with assigned test preparation and bacterial sampling (as described above) was followed by two additional treatments (at 2- to 3-h intervals). On days 3 and 4, all subjects washed their hands three times with chlorhexidine or alcohol at 2- to 3-h intervals, but no samples were taken. One wash was performed on day 5, and the samples were obtained for bacterial culture as described below.

Pour plates were prepared by using 1 ml of inoculum from each dilution (10^1 to 10^4). The plates were incubated at 35°C for 48 h. The number of viable bacteria recovered from the hand was determined by using the formula: (75 ÷ aliquot volume) × dilution factors × plate counts.

Diluent. Ten percent Trypticase soy broth containing 1% Tween 80 and 0.3% Azolectin (lecithin) was used as the diluent. Azolectin was the neutralizer for chlorhexidine and alcohol.

Trypticase soy agar containing 1% Tween 80 and 0.3% Azolectin was used. Standard sterile surgical gloves were prewashed with sterile, distilled water. The test preparations were 0.5% chlorhexidine gluconate alcohol emollient (HIBISTAT) and 70% isopropyl alcohol.

RESULTS

Base line comparison. The base line period was used to test for comparability of the two test groups and equality of the left- and right-hand bacterial counts.

The average bacterial counts expressed in $\log_{10}$, between left and right hands for each test group, are shown in Table 2. The difference for bacterial recovery between the left and right hands was not statistically significant ($P > 0.20$). The log number between the two groups (chlorhexidine and alcohol) are also shown in Table 2; the differences were not statistically significant ($P > 0.10$). The right hand was used to compare the immediate effect of the two preparations since it was sampled immediately after treatment, whereas the left hand (experimental) was used to test the effect of exposure time of the test preparation.

Immediate postwash comparison (immediate effect). Immediate antimicrobial effects were determined by sampling the right hands of all the test subjects for each treatment immediately after washing and comparing the base $\log_{10}$ values of the bacterial counts with $\log_{10}$ of the mean right-hand base line count.

When compared with the base line counts, significant downward trends were noted over the days for both preparations (Table 3). The percentage reductions of bacterial counts for immediate postwash on days 1, 2, and 5 were 85, 96, and 98%, respectively, for chlorhexidine treatment and 84, 93, and 90%, respectively, for alcohol treatment. The difference between the two treatments was not statistically significant on days 1 and 2 ($P > 0.10$), but was significant on day 5 ($P < 0.01$).

TABLE 2. *Base line average log means between right and left hands and between groups*

Hands	Chlorhexidine	Alcohol ($P > 0.10$)
Right (immediate effect)	6.3614	6.4322
Left (delayed effect)	6.3900	6.4731
$P^a > 0.20$		

[a] A two-sample t test was used to test the comparability of the $\log_{10}$ means of the two groups (chlorhexidine versus alcohol), and a paired t test was used to test the comparability of the $\log_{10}$ means of the right and left hands (immediate effect and delayed effect).

TABLE 3. *Comparison between immediate postwash*[a] *and base line $\log_{10}$ means*

Days	Chlorhexidine		Alcohol
1	4.9261 (85.2)	$P > 0.10$	5.2201 (84.1)
2	4.5240 (95.6)		4.799 (93.4)
5	4,1763 (98.2)	$P < 0.01$	4.8799 (90.3)

[a] The right hand was used to compare the immediate effects of the two preparations, since this hand was sampled immediately after washing.

[b] Numbers in parentheses indicate percentage reduction.

Delayed postwash comparison (persistent effect). The left-hand delayed postwash bacterial counts were used to determine the persistent activity of the test preparations. The $\log_{10}$ means for chlorhexidine and alcohol treatments for each exposure time and day are given in Table 4. The combined delayed postwash bacterial counts were significantly lower on days 1, 2, and 5 for both treatments when compared with the left-hand base line counts. The greatest reduction in bacterial counts was noted on the 5th day. The overall $\log_{10}$ means were 4.9943 and 5.4684 for chlorhexidine and alcohol, respectively. From the split-plot analysis of variance, the difference between the two treatments was statistically significant ($P < 0.05$). At no time did the delayed postwash bacterial counts approach the base line level.

DISCUSSION

Chlorhexidine has excellent in vitro antimicrobial activity not only against bacteria but also against several fungi (1, 6). Due to its broad-spectrum activities and negligible potential for skin reactions, chlorhexidine gluconate has become a widely used antimicrobial.

The present study demonstrated that the gloved-hand method for evaluating effects of antimicrobial agents on the microbial hand flora is a reproducible method for sampling skin flora. No significant differences in the number of bacteria between the right and left hands of individual subjects were observed ($P > 0.20$). The bacterial counts between the groups (chlorhexidine versus alcohol) during the base line study were not statistically significant ($P > 0.10$).

Microbial counts were rapidly reduced after immediate wash with both preparations. Although the reduction in bacterial counts was highly significant for all 3 test days, the differences between the two treatments were not significant on days 1 and 2. On day 5, the difference between chlorhexidine and alcohol treatments became significant ($P < 0.01$). When compared with the base line bacterial count, chlorhexidine treatment produced 98.2% reduction on day 5. There was a significant downward trend over days for both preparations ($P < 0.05$). We consider the immediate postwash results as indicative of excellent efficacy for both treatments.

The delayed postwash bacterial counts showed that there was no significant growth of bacteria over the 6-h period when compared with the base line counts. The average postwash bacterial counts in the chlorhexidine group were lower on day 2 than on day 1 and lowest on day 5. After 1 h, postwash bacterial counts showed an upward linear trend over different time exposures for both preparations.

The difference in log means between chlorhexidine (4.9943) and alcohol (5.4684) treatments was statistically significant ($P < 0.01$) when the average bacterial counts over all exposure times and days were compared.

In comparative studies, 0.5% chlorhexidine is a slightly better skin antiseptic than iodine and much better than 70% ethanol solutions (5, 7). Five percent chlorhexidine gluconate preparation was considered a superior antimicrobial agent to povidone-iodine and hexachlorophene (PHisoHex) when used as hand disinfectant (8, 9). Chlorhexidine gluconate produced the greatest initial reduction against resident flora, followed by povidone-iodine and hexachlorophene, respectively. On gloved hands, there was significant regrowth of resident flora after the use of povidone-iodine; there was no such significant regrowth on gloved hands after chlorhexidine and hexachlorophene treatments (9).

Our data indicate that both chlorhexidine and 70% alcohol were fast-acting antimicrobial agents. For substantive antimicrobial effect, chlorhexidine treatment produced statistically significant differences when compared with 70% alcohol treatment.

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TABLE 4. *Delayed postwash log means*[a]

Time interval (h)	Base line counts		Day 1		Day 2		Day 5	
	Chlorhexidine	Alcohol	Chlorhexidine	Alcohol	Chlorhexidine	Alcohol	Chlorhexidine	Alcohol
1	6.4306	6.4566	4.6553	5.5953	3.9129	4.3176	3.8265	4.8182
2	6.3188	6.3806	4.9611	5.6553	4.5126	5.4727	3.2499	5.1667
3	6.3905	6.5495	5.1912	5.4207	5.1308	5.1896	4.7632	4.8338
4	6.4599	6.3467	5.3361	5.7596	4.7727	5.6392	5.3041	5.5783
5	6.3920	6.6599	5.6757	6.0246	5.9086	5.6290	5.1103	6.000
6	6.3900	6.4735	6.1788	5.9561	5.7614	5.7627	5.3618	5.5518
Avg postwash $\log_{10}$ means	6.3900	6.4731	5.3421	5.7315	5.0088	5.3532	4.6137	5.3206

[a] Overall $\log_{10}$ means for all days and time intervals for chlorhexidine and alcohol were 4.9943 and 5.4684, respectively ($P < 0.05$, split-plot analysis of variance).

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