Population Kinetics of the Skin Flora on Gloved Hands Following Surgical Hand Disinfection With 3 Propanol-Based Hand Rubs: A Prospective, Randomized, Double-Blind Trial •
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Source: *Infection Control and Hospital Epidemiology*, Vol. 28, No. 3 (March 2007), pp. 346-350
Published by: The University of Chicago Press on behalf of The Society for Healthcare Epidemiology of America
Stable URL: http://www.jstor.org/stable/10.1086/510865
Accessed: 07/09/2013 03:25

ORIGINAL ARTICLE

Population Kinetics of the Skin Flora on Gloved Hands Following Surgical Hand Disinfection With 3 Propanol-Based Hand Rubs: A Prospective, Randomized, Double-Blind Trial

Manfred L. Rotter, MD; Günter Kampf, MD; Miranda Suchomel, DiplIng; Michael Kundi, PhD

OBJECTIVE. To study the bacterial population kinetics on gloved hands following hand treatment with 3 optically indistinguishable, alcohol-based surgical hand rubs, with and without supplements to delay bacterial regrowth.

DESIGN. Prospective, randomized, double-blind, balanced quasi–Greco-Latin square design.

SETTING. Microbiology laboratory of the Medical University Vienna, Austria.

PARTICIPANTS. Twenty-four healthy adult volunteers without skin lesions.

SURGICAL HAND RUBS. The following hand rubs, all stained blue, were applied to the hands for 3 minutes: 1-propanol 60% vol/vol (A); 2-propanol 70% m/m plus chlorhexidine gluconate 0.5% wt/wt (B); 2-propanol 45% wt/wt plus 1-propanol 30% wt/wt plus mecetronium etilsulfate 0.2% wt/wt (C). As a reference formulation (R), 1-propanol 60% vol/vol, unstained, was applied for the same amount of time.

METHOD. In 8 once-weekly tests, 24 subjects randomly assigned to use the 4 hand rubs in groups of 6 persons each performed hand hygiene according to the method described in European Norm 12791. Every subject used one preparation at a time, the antimicrobial effect of which was evaluated at 2 sampling times. After week 8, each volunteer had tested every preparation at every preset sampling time. All preparations were tested in parallel.

RESULTS. The mean pretreatment counts of viable bacteria (in colony-forming units per milliliter) in fluid samples were not significantly different between week 1 and week 8, nor between the right and left hands (analysis of variance [ANOVA], $P > .1$). Immediately after applying the formulation (t_0), bactericidal effects of the blinded formulations A and C were equivalent to that of the reference formulation R, whereas the effect of B was questionable. The population kinetics of the flora on the hands proceeded from large and fast initial reductions of the skin flora by 2.7 log units (A), 3.1 log units (B), 3.3 log units (reference formulation), and 3.5 log units (C), to slow regrowth. However, even after 6 hours wearing gloves viable bacterial counts remained significantly ($P < .01$) below the baseline values (by 0.9 log [reference formulation], 1.1 log [A and B], and 1.5 log [C]). The slowest regrowth 1 and 3 hours after application (Δ from t_0, 0.1 log and 0.7 log respectively) was seen with formulation C, and the slowest regrowth after 6 hours was seen with formulation B (Δ from t_0, 1.6 log). These differences did, however, not reach statistical significance.

CONCLUSIONS. With respect to the rapid and dramatic antibacterial action of suitable alcohols at high concentrations and with appropriate neutralizers, the contribution of supplements to the delay of bacterial regrowth on gloved hands appears rather minor, if a product only exerts an immediate effect equivalent to that of the reference disinfection procedure described in EN 12791.

Infect Control Hosp Epidemiol 2007; 28:346-350

Surgical hand disinfection has become an infection control standard worldwide.[1] Its aim is the reduction of the release of resident and transient microbial flora on the hands into surgeons' gloves.[2] Given an average glove perforation frequency of 18%, the immediate and persistent effects of surgical hand rubs have become important quality markers for preoperative hand preparation.[3] It is, therefore, of utmost interest to identify a surgical hand rub that has maximum bactericidal efficacy on both transient and resident hand flora. Because of their wider antimicrobial spectrum,[4-7] faster action,[7,8] and better skin tolerability,[7,9-13] alcohol-based hand rubs are often recommended[1,9,14] and preferred to antimicrobial soaps containing chlorhexidine gluconate (CHG) or povidone iodine.

A definition for persistent effect with respect to hand hygiene has recently been suggested, [15] but the necessary du-

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Received December 1, 2005; accepted May 8, 2006; electronically published February 15, 2007.

 0899-823X/2007/2803-0015$15.00. DOI: 10.1086/510865

ration of this effect remains a matter for discussion.[2,15] In model described in the European test standard European Norm (EN) 12791, the persistent effect is measured after surgical gloves have been worn for 3 hours,[16] whereas, according to the tentative US Food and Drug Administration test method, it is assessed after 6 hours.[17] Although the duration of most surgical procedures does not exceed 3 hours,[18] there are currently no data to demonstrate whether 3 or 6 hours is most valid for measurement of persistent effect. The main aim of surgical hand disinfection is to keep the bacterial density on the hands below baseline for the duration of an operation.[15] In EN 12791, the antibacterial effect is assessed only twice (immediately after disinfection and 3 hours later). Hence, this method does not include the assessment of the effect at 6 hours. More measurements at various times would certainly be helpful for obtaining more information about the kinetics of bacterial regrowth on gloved hands.

The new Centers for Disease Control and Prevention guideline for hand hygiene recommends the use of either an antimicrobial soap or an alcohol-based hand rub supplemented with agents conferring a persistent effect for surgical hand antisepsis.[1] The agent most commonly thought to offer this persistent type of effect is CHG.[19] However, although often claimed, this effect has, to the best of our knowledge, never been adequately confirmed in a randomized, double-blind, controlled trial. Furthermore, recent data suggest that it might also be explained by the insufficiently neutralized bacteriostatic activity of CHG that is carried over with sampling fluids onto counting plates.[20] Indeed, it is well-known that effective neutralization of CHG is difficult to achieve.[7,21,22] Therefore, CHG might cause a more persistent bacteriostatic effect than would otherwise be seen with compounds that are more easily neutralized.

With the above in mind, we undertook a prospective, randomized, double-blind trial to study the immediate and persistent effects of surgical hand rubs applied for 3 minutes, with special consideration of the bacterial population kinetics on gloved hands. For this study , we measured the release of skin flora from the hands at hours 0, 1, 3, and 6 (t_0, t_1, t_3, and t_6) after disinfection with 3 different alcohol-based formulations. One preparation consisted only of alcohol, the others contained either CHG or mecetronium etilsulfate (MES) as supplements, which are thought to confer a persistent effect. In addition, 1-propanol (60% vol/vol) was used for a reference formulation in the disinfection procedure described in EN 12791.

METHODS

The following hand-rub preparations were used: (A) 1-propanol, 60% vol/vol; (B) 2-propanol (70% wt/wt) plus CHG (0.5% wt/wt) (Hibistat, Regent Medical); and (C) 2-propanol (45% wt/wt), 1-propanol (30% g/g) and mecetronium etilsulfate (MES) (0.2% wt/wt) (Sterillium, Bode). For blinding, the products were labeled as solutions A, B, and C and stained blue so that they appeared the same. In contrast, the reference formulation, 1-propanol, was left unstained.

Twenty-four volunteers were included in the trial. Exclusion criteria were age of 18 years or less, pregnancy, and the presence of skin breaks such as cuts, abrasions, or other skin disorders on the hands. Nails were kept short and clean and the volunteers agreed not to ingest or use any antibacterial substance during the trial, starting 1 week prior to testing. All participants gave their written informed consent.

Media used were those described in EN 12791. Sampling and dilution solutions used tryptic soy broth (Caso broth, Merck). Counting plates used tryptic soy agar (TSA) (Caso agar, Merck)

The neutralizing agent contained in the sampling fluids and their diluents (but not in the counting plates) used for assessing posttreatment bacterial counts was a mixture of 3% Tween 80 (Merck), 3% saponin (Riedel-deHaen), 0.1% L-histidine hydrochloride (Merck) and L-cysteine (Merck). In preceding tests, this neutralizer was found to be the most active.

A dilution (20%) of nonmedicated, sterile soft soap (Apoca) was used for pretest hand cleansing.

A total of 8 test runs were performed, at intervals of 1 week. Twenty-four volunteers were randomly assigned by computer to use 1 of 4 hand rubs, including the reference formulation (4 groups of 6 persons), and subgroups were randomized to use the 4 sampling times (0, 1, 3, and 6 hours), such that, using the split-hands model,[23] for each test run a volunteer used 1 preparation that was evaluated at 2 different sampling times. After week 8, each volunteer had tested every preparation at every predetermined sampling time. All preparations were tested in parallel. To guarantee balance of the sequence of tests, the 4 groups, 4 preparations, and 4 sampling times were arranged in a quasi–Greco-Latin square under the constraint that the sampling times were not equal for both hands. The Greek letters were interpreted as applying to the left hand; the Latin letters applied to the right hand. In contrast to a true Greco-Latin square design, however, each subject eventually tested every preparation at all 4 sampling times. This was accomplished by a pair of Greco-Latin squares that defined a sequence of 8 experimental conditions for each group, with 2 sampling times on each experimental day.

The test method was that described in EN 12791, using 24 rather than 20 volunteers and including 2 additional sampling times. Pretreatment values were established by rubbing and kneading the fingertips for 1 minute at the bottom of a Petri dish—one for each hand—in 10 mL of tryptic soy broth without neutralizer. Subsequently, one of the surgical hand rubs was applied using the standardized rub procedure described in EN 12791: 3 mL of the hand rub was poured into the cupped hands, distributed over the skin surface up to the wrists, and vigorously rubbed into the skin. As many 3-mL portions were applied as were necessary to keep the hands wet for a total of 3 minutes. After the end of disinfection, the fingertips were sampled according to the randomized

sampling times assigned, either for immediate (t_0) posttreatment values or—after air-drying and gloving—for later posttreatment values (t_1, t_3, or t_6) values. At these testing times, the sampling fluids and their diluents contained neutralizer.

Quantitative surface cultures of sampling fluids and their dilutions were done on TSA. As described in EN 12791, counting plates were incubated at 36°C; colonies were counted after 18 to 24 hours and again after incubation for a further 24 hours.

For statistical evaluation, viable bacterial counts were processed as described in EN 12791. Bacterial reduction factors (RFs) were expressed as the difference between the log pretest value and the log posttest value, per person and hand. RF is the ratio of pretest to posttest values (in cfu/mL) and is expressed by its decimal logarithmic value as log RF.

To ensure that intraindividual baseline conditions were comparable between left and right hands and throughout the 8 weeks of the trial, analysis of variance (ANOVA) for repeated measurement was performed for the log pretest values with the factors "hands" (left or right) and "weeks" (1 through 8). The α was set at 10%; the level of significance was set high in order not to overlook a potential difference that could affect the results of the tests for the different preparations. Mean log RFs derived from measurements at t_0 for formulations A, B, or C were compared with that of reference formulation R by means of equivalence testing applying exact 90% confidence limits according to Hodges and Lehmann[24] with a safety margin of 0.6 log. Mean RFs for formulations A, B, or C derived from samples obtained at t_1, t_3, or t_6 were tested for significant differences to the corresponding means for reference formulation R, first by the nonparametric Friedmann ANOVA and subsequently in pair-wise post hoc comparisons by Wilcoxon-Wilcox-tests at $P = .01$ (one-sided). The log posttest values at t_6 were tested against the corresponding log pretest values by Wilcoxon matched-pairs tests.

RESULTS

As indicated by the results of ANOVA (not shown), there were neither significant differences between the mean log pretest values for the left and right hands, nor between the log pretest values assessed during weeks 1 to 8.

The results of equivalence testing (not shown) revealed that the immediate in vivo bactericidal effects of a 3-minute application of preparations A and C were clearly equivalent to those of the reference formulation R, whereas the effect of formulation B was questionable.

As seen in the Table, at t_0 the reference disinfection formulation R had reduced bacterial release from the fingertips by 3.3 ± 1.0 log. Even 6 hours later, the mean bacterial density in the sampling fluids still remained 0.9 log less than the baseline value ($P < .01$). Similarly, blinded use of preparation A (n-propanol 60%) yielded comparable results at each sampling time. Preparation B (2-propanol 70% plus CHG 0.5%) was somewhat less effective, especially at t_0, though never significantly. Preparation C (2-propanol 45%, 1-propanol 30%, plus MES 0.2%) proved to be the most efficacious disinfectant at all sampling times; the differences in mean RF values reached significance ($P \leqslant .01$) when compared with those of reference formulation R at t_3 and with those of formulation B at t_1 and t_3. Six hours after disinfection, bacterial densities with all preparations were still significantly ($P < .01$) less than the baseline values.

Bacterial population kinetics on gloved hands can be described as follows: after a very large and fast reduction of bacterial release from the fingertips (mean RFs, 2.7-3.5 log), a slow regrowth of the skin flora is indicated by decreasing mean log RFs which, however, do not reach 0 (ie, the baseline value) even after 6 hours. The slowest regrowth at 1 and 3 hours after application was seen with formulation C (change from $t_0 = 0.1$ log and 0.7 log, respectively). After 6 hours, the slowest regrowth was observed with formulation B (change from $t_0 = 1.6$ log). Thus, with the preparations containing a nonvolatile active agent, a somewhat, though not significantly, slower regrowth of resident hand flora was seen, compared with the rubs A and R, which contained solely 1-propanol.

TABLE. Effects of 3-Minute Applications of Surgical Hand Rubs With 3 Alcohol-Based Preparations (A, B, C) and a Reference Disinfection Formulation (R) on the Microbial Population Kinetics of Gloved Hands

Preparation	Active ingredient(s)	Mean log reduction factor ± SD, by time after application			
		Hour 0	Hour 1	Hour 3	Hour 6
A	1-propanol 60% vol/vol	3.1 ± 0.9	2.9 ± 1.2	2.2 ± 0.9	1.1[a] ± 1.0[b]
B	2-propanol 70% + CHG 0.5% wt/wt	2.7 ± 1.2	2.3 ± 1.1[c]	1.7 ± 1.2[c]	1.1 ± 0.8[b]
C	1-propanol 30% + 2-propanol 45% + MES 0.2% wt/wt	3.5 ± 1.2	3.4 ± 1.1	2.8 ± 1.3[d]	1.5[a] ± 1.0[b]
R	1-propanol 60% vol/vol	3.3 ± 1.0	2.6 ± 1.3	1.8[a] ± 0.8	0.9[a] ± 0.7[b]

NOTE. CHG, chlorhexidine gluconate; MES, mecetronium etilsulfate. For definition of reduction factor (RF), see Methods.
[a] $P \leqslant .01$ vs corresponding hour 3 value.
[b] $P \leqslant .01$ vs baseline value.
[c] $P \leqslant .01$ vs formulation C.
[d] $P \leqslant .01$ vs reference formulation R.

DISCUSSION

For surgical hand disinfection, a fast and large immediate antimicrobial effect is desired to enable the surgical team to begin their work without delay and with clean hands. In addition, reduced bacterial release should last for the duration of an operation to help ensure that, in case of glove damage, a potential microbial inoculum in the wound is less than an infection-generating dose. Lacking epidemiological data on the magnitude of microbial reduction on the hands necessary to prevent infection—which is probably quite variable—it has arbitrarily been established in EN 12791 that the effect of a surgical scrub should at least be equivalent to a 3-minute application of hand rub containing 1-propanol 60% vol/vol.

To the best of our knowledge, this is the first prospective, randomized, double-blind trial to generate detailed information on the population kinetics of skin flora on gloved hands subsequent to surgical scrubbing with alcohol-based hand rubs with or without supplements believed to delay bacterial regrowth.

The immediate antimicrobial effect of the 4 preparations, as achieved with 3-minute applications, was fast and large. This is probably entirely because of the alcohols, the activity of which (up to a certain limit) is positively associated with their concentration and with the type of alcohol; 1-propanol is the most effective.[19,25] Therefore, it is not surprising that preparation C (2-propanol 45%, 1-propanol 30% plus MES 0.2%), with its high total alcohol concentration of 75% (wt/wt) and 1-propanol constituting 30% of the mixture, causes the strongest reduction. This confirms the findings of other investigators who have reported that, among 5 surgical hand rubs, formulation C was the only one more efficacious than the reference rub.[26] In addition, it was reported recently that for hand rub C, an application time of only 1.5 minutes was still enough to exceed the efficacy of a 3-minute application of the reference disinfection formulation, which confirms the superior performance of formulation C.[27] In contrast, a 70% wt/wt 2-propanol–based rub is neither as effective as formulation C nor as 1-propanol formulations A and R at their concentration of 60% vol/vol (approximately 53% wt/wt). Indeed, whereas the antimicrobial effects of both formulations A and C were found to be equivalent to those of reference formulation R, those of formulation B were questionable. Also, if tested in a paired fashion, the activity of formulation B was significantly inferior to that of formulation C (Table). Hence, there exist and there have been reported significant differences in the antimicrobial efficacy of alcohol-based products.[26,27] Testing of new products is, therefore, necessary and justified, even if the active ingredients and their concentrations are known.

With regard to the effects of the hand rubs at 1, 3, and 6 hours after application, it is interesting to note that preparations B and C (containing CHG and MES, respectively) did cause some persistent effects, but in this study, they were not significant. A statement such as this must, however, be made with caution because the effects of both formulations were not compared with those of identical formulations lacking CHG or MES. This may limit the validity of conclusions about these preparations. Bacterial regrowth was slowest 1 and 3 hours after application with formulation C (containing MES), and after 6 hours with formulation B (containing CHG). Thus, it seems that MES and CHG do cause a certain persistent effect; however, it appears to be smaller than expected and reported earlier.[19] This may be explained by more effective neutralization of CHG and MES in this study. Indeed, it has been shown that in other studies, ineffective neutralization may have produced false positive efficacy data.[20]

It is noteworthy that even 6 hours after disinfection, the bacterial release from the fingertips was still significantly ($P < .01$) less than baseline levels, by 0.9 to 1.5 log. This signifies that bacterial regrowth on gloved hands obviously takes more than 6 hours to reach baseline levels, as long as the skin flora has been reduced substantially enough at t_0, as it was in the case with use of the alcohols alone in this study. Hence, from the results obtained with the 2 pure 1-propanol formulations (A and R), it may be concluded that a persistent effect may not be necessary for surgical hand rubs if they exert an immediate antimicrobial effect that is at least equivalent to that of the reference disinfection formulation described in EN 12791. Therefore, with respect to the fast and strong action of suitable alcohols at high concentrations, the contribution of supplements to delay regrowth of skin flora appears rather minor.

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