Antimicrobial Activity of Chlorhexidine Gluconate against Natural and Artificial Contamination during Simulation of In-Use Conditions

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Abstract □ Commercial bottles containing chlorhexidine gluconate (antimicrobial skin cleanser), whether exposed to room atmosphere or experimentally inoculated with *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Pseudomonas cepacia*, and *Proteus mirabilis*, did not exhibit microbial growth.

Keyphrases □ Chlorhexidine gluconate—antimicrobial activity, simulation of natural and artificial contamination □ Antimicrobial agents—chlorhexidine gluconate, simulation of contamination □ Skin cleanser—chlorhexidine gluconate, antimicrobial activity, simulation of contamination

Chlorhexidine gluconate has broad-spectrum antimicrobial activity against bacteria and several fungi (1–3). Although the antimicrobial activity of chlorhexidine when used on human skin is well documented, knowledge about bacterial contamination of commercial preparations during in-use conditions is lacking (4–6).

The present study was designed to determine the potential for bacterial contamination of a commercial chlorhexidine gluconate[1] skin cleanser by repeated exposure of opened bottles to room atmosphere over a prolonged period and to determine the potential for survival or growth of bacteria after repeated bacterial challenges with *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Pseudomonas cepacia*, and *Proteus mirabilis*. The test organisms were identified by cultural and biochemical reactions (7). Bottle-to-bottle transfers of the preparation were made to reflect certain in-use situations where residual product left in a used bottle is mixed with fresh product.

EXPERIMENTAL

Environmental Contamination—Standard commercial bottles containing ~946 ml of the antimicrobial skin cleanser, tested and found free of contamination, were exposed to room atmosphere for 6 months by removing the tops daily and shaking for 5 min. Samples were taken weekly for the first 2 months and then at monthly intervals.

Two methods were utilized for determining bacterial contamination:

1. A 1-ml sample from each bottle was removed, diluted in trypticase soy broth containing 10% polysorbate 80[2] and 3% soy phosphatides[3] (neutralizer), and cultured on tryptic soy agar (containing 1% neutralizer). Culture plates were incubated at 37° for 48 hr.

2. A 20-ml aliquot was mixed with 250 ml of trypticase soy broth containing 0.3% neutralizer and then passed through a 0.45-μm filter disk. One-half of the filter was transferred to trypticase soy broth and the other half to thioglycollate broth. After 24 hr of incubation at 37°, 1 ml of each broth was cultured on tryptic soy agar.

Experimental Contamination with *S. aureus*, *P. mirabilis*, *Ps. aeruginosa*, and *Ps. cepacia*—For a given test organism, the experimental contamination procedure comprised seven bacterial challenges and three bottle-to-bottle transfers over 25 weeks. A commercial bottle (Bottle 1) of the chlorhexidine gluconate preparation, previously tested and found to be free of bacteria, was challenged (C-1). The bottle contents were mixed, and the aliquots were tested at 10 min and after 1 week (just before C-2) for bacterial growth. Aliquots (25 ml) were removed *via* the delivery spout of the bottle, employing a hand pump to simulate in-use conditions, and were cultured for environmental contamination. The bottle then was challenged as before (C-2, 1 week). The aliquots were tested at 10 min and after 1 week (just before C-3) for bacterial growth. This contamination and testing for bacterial growth were repeated at 2 (C-3) and 3 (C-4) weeks. Challenge 5 (4 weeks) was performed as described, and the aliquot was tested for bacterial growth at 10 min only.

At this point, periodic bottle-to-bottle transfers of the preparations were begun to mimic certain in-use conditions, where fresh product was poured into a previously used bottle containing residual product with 1 ml containing 10^6 viable bacteria (for a final concentration of 10^3 bacteria/ml in the commercial bottle).

First Transfer (4 Weeks)—Forty milliliters from Bottle 1 was transferred aseptically into a new bottle (Bottle 2), previously tested and found free of bacteria. The aliquot was tested after 1 week (just before C-6) for bacterial growth. Bottle 2 was challenged (C-6, 5 weeks). The aliquot was tested at 10 min and also after 4 weeks (just before the second bottle-to-bottle transfer) for bacterial growth.

Second Transfer (9 Weeks)—Forty milliliters from Bottle 2 was transferred aseptically to a new bottle (Bottle 3). The aliquot was tested after 4 weeks (just before C-7) for bacterial growth. Bottle 3 was challenged (C-7, 13 weeks). The aliquot was tested at 10 min and also after 4 weeks (just before the third bottle-to-bottle transfer) for bacterial growth.

Third Transfer (17 Weeks)—Forty milliliters from Bottle 3 was transferred to a new bottle (Bottle 4). The aliquot was tested for bacterial growth after 8 weeks, concluding a total period of 25 weeks from the initial challenge.

RESULTS AND DISCUSSION

No contamination was noted at the start of the study or after 5-min daily exposures and shaking of the product at room atmosphere for the 6-month duration of the environmental contamination. Delivery spouts of the bottles were cultured with a swab and were free of bacterial contamination.

Bottles of the products contaminated experimentally with *S. aureus*, *P. mirabilis*, *Ps. aeruginosa*, or *Ps. cepacia* were examined for 6 months for bacterial growth and were not contaminated. Delivery spouts also were free of bacterial contamination.

Chlorhexidine has excellent antimicrobial activity against Gram-positive and Gram-negative bacteria (1–6). Due to its broad-spectrum antimicrobial activities, chlorhexidine preparations have become widely used for disinfection.

[1] Hibiclins, ICI America.
[2] Tween 80, Sigma Chemical Co.
[3] Asolectin, Woodside, N.Y.

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