ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1991, p. 627–631
0066-4804/91/040627-05$02.00/0

Vol. 35, No. 4

Effect of Topical Antimicrobial Treatment on Aerobic Bacteria in the Stratum Corneum of Human Skin

J. OWEN HENDLEY* AND KATHLEEN M. ASHE

Department of Pediatrics, University of Virginia Health Sciences Center, Box 386, Charlottesville, Virginia 22908

Received 27 August 1990/Accepted 23 January 1991

The efficacy of antimicrobial agents applied topically to the skin surface in eradicating coagulase-negative staphylococci (CNS) residing in the stratum corneum underlying the surface was examined. Glabrous skin was sampled with a 26-cm^2 contact plate containing Trypticase soy agar. Five antiseptic solutions and four antimicrobial ointments were evaluated. The antiseptic solutions (10% povidone-iodine, 2% aqueous iodine, 2% tincture of iodine, 70% ethanol, and 0.5% chlorhexidine-ethanol) were applied for 15 s with a gauze sponge. The antimicrobial ointments (iodophor, silver sulfadiazine, mupirocin, and a triple-antibiotic ointment containing neomycin, polymyxin, and bacitracin) were applied and covered for 6 h with gauze. After treatment, the surface was sampled, 15 to 25 keratinized layers were subsequently removed by sequential stripping with cellophane tape, and the stratum corneum was sampled. All agents were effective in eradicating CNS from the surface (80 of 88 trials). However, only 2% iodine (17 of 20 trials), iodophor (8 of 12), mupirocin (6 of 10), and the triple-antibiotic ointment (9 of 11) eradicated CNS from the stratum corneum reliably (≥50% of trials). The stratum corneum was repopulated with resident flora within 24 h of treatment with 2% iodine (4 of 4 trials), iodophor (6 of 7), or mupirocin (5 of 6), but repopulation occurred in only 1 of 7 trials with the triple-antibiotic ointment. Topical treatment of skin with antimicrobial agents usually eradicates CNS from the skin surface but may not eradicate CNS from the stratum corneum. Only the triple-antibiotic ointment eradicated CNS from the stratum corneum and prevented repopulation with resident flora.

Coagulase-negative staphylococci (CNS), which frequently cause incision site and device-related infections, may be derived from the normal resident flora of the skin. We examined the microbial anatomy of the normal human epidermis by characterizing CNS on the surface of normal skin and in the keratinized stratum corneum beneath the surface (3). Removal of the top 5 keratinized layers of the epidermis at a site by stripping with cellophane tape resulted in an 80% reduction in the number of colonies detected; the number of colonies then remained constant during the removal of 20 additional keratinized layers (3). This result implied that CNS were evenly distributed in the stratum corneum. After a site in the stratum corneum was sterilized by the application of ethanol or tincture of iodine, CNS repopulated the site within hours (3). The repopulation phenomenon suggested that there was a reservoir of aerobic bacteria beneath the skin surface which was maintaining resident flora. The presence of this reservoir may have implications for devices left in place after surgical insertion through the epidermis following sterilization of the surface.

In the present study, the efficacy of antimicrobial agents applied to the skin surface in eradicating bacteria both on the surface and in the underlying stratum corneum was examined. Most antimicrobial agents effectively eradicated bacteria from the surface but not from the stratum corneum. Surprisingly, one ointment containing three antibiotics (neomycin, polymyxin, and bacitracin) not only eradicated organisms on the skin surface and in the stratum corneum but also appeared to prevent overnight repopulation with CNS from the deeper reservoir.

* Corresponding author.

MATERIALS AND METHODS

Skin sampling. Various skin sites relatively free of terminal hair (such as the upper chest, upper back, volar surfaces of the arms, upper thighs, and abdomen) were sampled on 14 healthy adult volunteers who gave written informed consent. Subjects were of any race or gender, and none had used topical or systemic antimicrobial agents in the week prior to our study. An individual skin site was not sampled more frequently than once per week so that the skin could return to its "normal" state (2, 6). Sites with little hair were chosen so that tape stripping would not be so uncomfortable.

A template of flexible plastic (14 by 11 cm, 0.5 mm thick) with a center hole the size of a contact plate was devised to facilitate repetitive sampling of precisely the same site. The skin was marked along the outside edges of the template so that the template could be returned to the same position after treatment or tape stripping. The skin surface was washed with Ivory bar soap and water and allowed to air dry prior to sampling. A 26-cm^2 area was sampled through the hole in the template by applying a contact plate (Rodac; Falcon, Oxnard, Calif.) containing Trypticase soy agar (BBL Microbiology Systems, Inc., Cockeysville, Md.) to the skin with firm pressure. The template was wiped with 70% ethanol and air dried between samplings to prevent carry-over contamination.

The aerobic flora in the stratum corneum beneath the surface was examined with the cellophane tape stripping method described by Pinkus (9) and used previously in our laboratory (3). Three-inch (ca. 7.6-cm)-wide clear cellophane tape (Scotch; 3M Co., St. Paul, Minn.) was applied to the skin and stripped off to remove a layer of keratinized epithelium. Several unused areas of the sticky side of the cellophane tape were cultured with the contact plates and found to be free of CNS. After the removal of up to 25 layers

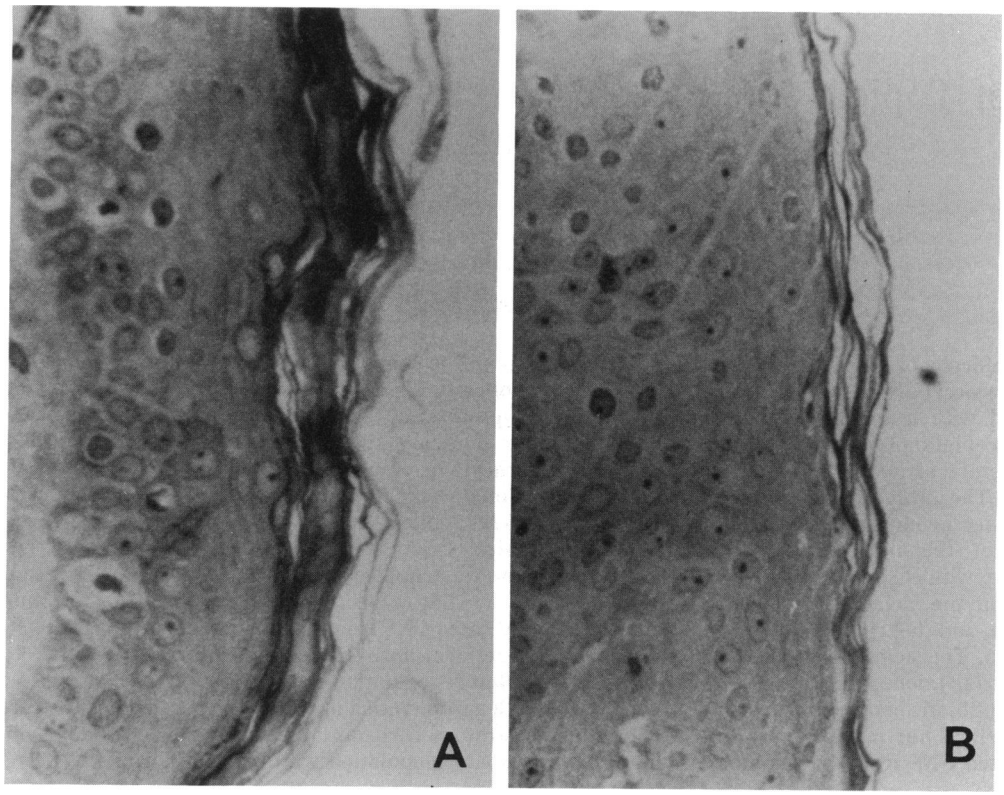

FIG. 1. Sections of adjacent areas of skin depicting the effect of stripping with cellophane tape on the epidermis. (A) After a soap and water wash; no stripping. (B) After the removal of 25 layers by stripping. Toluidine blue stain. Original magnification, ×100.

by stripping, the site was recultured with the template as a guide.

The effect of tape stripping on the epidermis was visualized by biopsy of the skin on the midback of one investigator (J.O.H.). Two adjacent areas on the skin were washed with soap and water, one of the areas was stripped 25 times with cellophane tape, and a biopsy specimen from each area was obtained with a 3-mm punch. Both biopsy specimens were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) and embedded in Epon 812 (EMS, Fort Washington, Pa.). Sections (0.5 μm) were stained with 0.5% toluidine blue in 0.5% aqueous sodium borate for examination by light microscopy. Twenty-five tape strippings resulted in the removal of part, but not all, of the stratum corneum (Fig. 1). The level of the epidermis exposed was still in the keratinized cell layers and not in the stratum granulosum.

Inoculated contact plates were incubated in an aerobic atmosphere at 37°C for 24 h before colonies were counted. The site was defined as sterile or sterilized if no bacteria grew on the agar surface after 48 h. The numbers of colonies were quantitated visually by 24 h, when they were small enough to be counted accurately. The grid on the back of the contact plate was used to subdivide the plate into 1- by 1-cm grid boxes. The colonies in each grid box were counted individually, and the totals of all grid boxes on the plate were added together. One or two colonies located at the extreme edge of the contact plate surface were presumed to be contaminants introduced by handling of the plate and were not counted.

Antimicrobial agents. Five liquid antiseptics were tested: 10% povidone-iodine (Betadine; Purdue-Frederick Co., Norwalk, Conn.), 2% aqueous iodine in 4% potassium iodide, 2% tincture of iodine (Humco Laboratories, Texarkana, Tex.), 70% ethanol, and 0.5% chlorhexidine acetate (Sigma Chemical Co., St. Louis, Mo.) in 70% ethanol. Control sites for the antiseptics were not treated after the initial soap and water wash. The four antimicrobial agents tested were in the form of topical ointments: 1% silver sulfadiazine (Silvadene Cream; Marion Laboratories, Kansas City, Mo.), an iodophor containing 1% povidone-iodine (Operand; Redi Products, Prichard, W.Va.), 2% mupirocin (Bactroban; Beecham Laboratories, Bristol, Tenn.), and a triple-antibiotic ointment containing neomycin, polymyxin B sulfate, and bacitracin (Triomycin; Thames Pharmacal Co. Inc., Ronkonkoma, N.Y.). Control sites for the ointments were treated with white petrolatum (Pharmaderm, Melville, N.Y.), which was the vehicle for all of the ointments except mupirocin.

Skin treatment. After the skin surface was sampled, the site was treated with one of the nine antimicrobial agents. Each agent was tested on skin sites on at least four subjects. Each antiseptic solution, applied to a gauze sponge, was rubbed onto the skin for 15 s. The treated site was air dried before the surface and stratum corneum were sampled. Each ointment was applied to the skin with a sterile, gloved finger. The site was covered with sterile gauze for 6 h before the surface and underlying stratum corneum were sampled (after the gauze was removed).

Repopulation of treated sites. Some of the skin sites at which the surface had been treated with tincture of iodine or one of the ointments (mupirocin, iodophor, triple antibiotic) were examined to determine whether repopulation from a deeper reservoir would occur. After the stratum corneum at a site had been sampled 15 or 25 layers deep, the site was

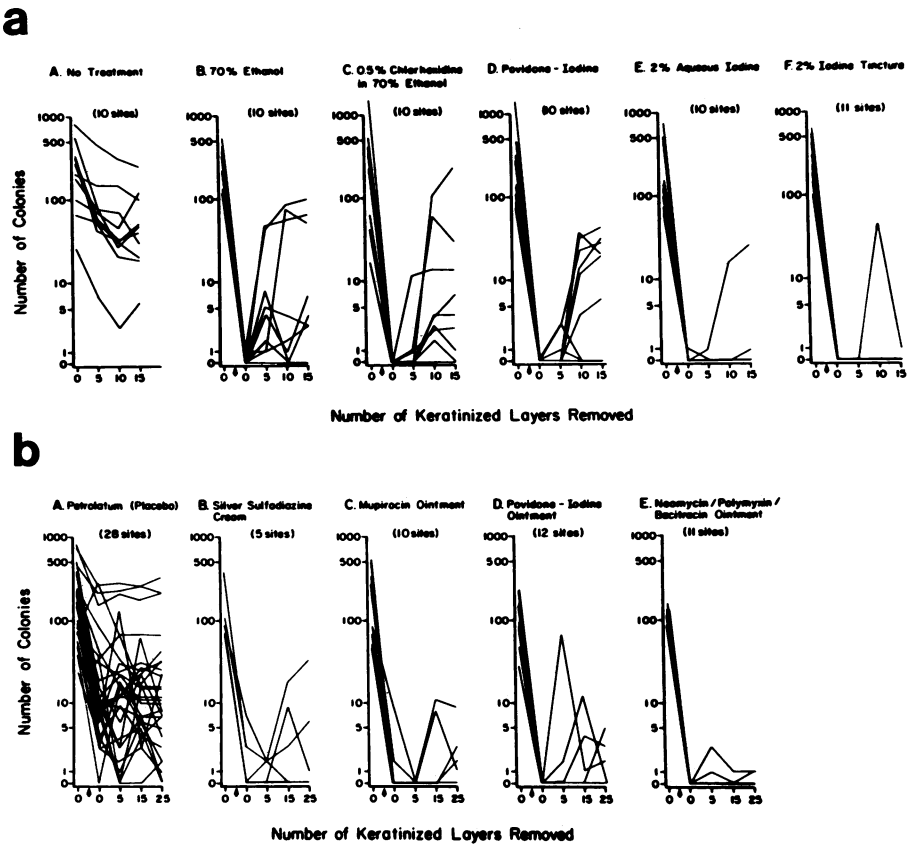

FIG. 2. Effect of antimicrobial treatment of the skin on aerobic flora on the skin and in the stratum corneum. (a) Antiseptic solutions. (b) Antimicrobial ointments. The arrow denotes the application of the antiseptic or ointment to the skin surface.

covered with a sterile dressing for 16 to 24 h. The dressing was removed, and the site was sampled both directly and after the removal of five additional keratinized layers.

Statistics. Fisher's exact test (two-tailed) was used for the comparison of eradication rates by agents. A comparison with a P value of >0.05 was not considered significantly different.

RESULTS

Sterilization of the surface. The number of colonies detected on the skin surface sampled with a 26-cm^2 contact plate ranged from 17 to more than 1,000 following the soap and water wash. Most samples contained from 100 to 500 colonies (mean, 234; median, 157). The skin surface was effectively sterilized with eight of the nine antimicrobial agents tested (Fig. 2). The antiseptic solutions povidone-iodine, chlorhexidine acetate in 70% ethanol, and 2% tincture of iodine eradicated surface bacteria in every trial (10 of 10 each), and 2% aqueous iodine and 70% ethanol eradicated bacteria from the surface in 9 and 8 of 10 trials, respectively. In contrast, none of the 10 control sites was sterile ($P < 0.001$).

Three of the four antimicrobial ointments were also effective in the sterilization of the skin surface; 1% silver sulfadiazine was the exception (Fig. 2b, graph B). Aerobic bacteria were eradicated from the skin surface at all sites treated with iodophor (12 of 12) and triple-antibiotic (11 of 11) ointments and at 8 of 10 sites treated with mupirocin ointment ($P < 0.001$, compared with the control), but only 2 of 5 1% silver sulfadiazine-treated sites (40%) were sterile (not significant). One of the 28 control sites (4%) was sterile.

Sterilization of the stratum corneum. Whereas treatment with eight of the antimicrobial agents usually sterilized the skin surface, eradication of bacteria from the stratum corneum under the sterilized surface varied with the agent used (Fig. 2). The rates of eradication from the stratum corneum after surface treatment with 70% ethanol (2 of 9 sites), chlorhexidine-ethanol (2 of 10), and povidone-iodine (4 of 10) were not statistically different from those for the control sites. On the other hand, bacteria in the stratum corneum were eradicated from most, but not all, of the sites after surface treatment with aqueous iodine (8 of 12 sites), tincture of iodine (9 of 10), mupirocin ointment (6 of 10), povidone-iodine ointment (8 of 12), and triple-antibiotic ointment (9 of 11) ($P \leq 0.001$ for each treated site versus control or untreated site). The stratum corneum was not sterile at any of the 10 untreated sites (Fig. 2a, graph A) or any of the 28 petrolatum-treated control sites (Fig. 2b, graph A).

Repopulation. Repopulation of some treated sites was studied to determine whether surface (topical) treatment could eradicate bacteria from the reservoir(s) which maintains the resident flora of the epidermis. Bacteria persisted but did not increase in number at the 13 placebo-treated sites (Fig. 3). Repopulation or persistence of bacteria occurred at all four sites treated with tincture of iodine, five of six mupirocin-treated sites, and six of seven iodophor-treated sites. In contrast, six of seven sites treated with the triple-antibiotic ointment (86%) were not repopulated with bacteria

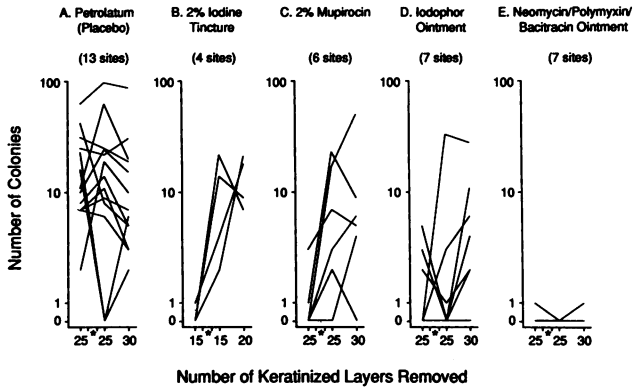

FIG. 3. Repopulation of the stratum corneum covered for 16 h with a sterile dressing after antimicrobial treatment of the skin surface. The skin surface was treated, 15 or 25 keratinized layers were removed, and the site was sampled, covered with a sterile dressing for 16 h (denoted by the asterisk), and resampled before and after the removal of 5 additional keratinized layers.

overnight ($P < 0.05$ for triple-antibiotic ointment versus each of the other treatments; Fisher's exact test). Moreover, the single site which was not sterile after being covered overnight had only one colony detected after the removal of five keratinized layers (Fig. 3E).

DISCUSSION

In the initial investigation of the microbial anatomy of normal human skin in our laboratory (3), the plasmid patterns of CNS isolated from the surface and stratum corneum of skin sites were used as epidemiological markers. The findings in that study suggested that CNS on the skin surface were derived from many sources, while CNS in the stratum corneum represented the indigenous resident flora replenished from a deep reservoir. Earlier work by Lovell (5), Montes and Wilborn (8), and Selwyn and Ellis (10) had suggested that the reservoir for the resident flora of glabrous skin was in the sebaceous glands and associated hair follicles in the dermis.

In the present study, the effect of antimicrobial treatment on the resident flora at a skin site was examined. Eight of nine antimicrobial agents eradicated bacteria from the skin surface, but eradication from the stratum corneum following treatment of the surface was variable. Ethanol, ethanol-chlorhexidine, and 10% povidone-iodine solutions were not effective, whereas iodine solutions and ointments containing mupirocin, iodophor, and three antibiotics were effective in eradicating organisms from the stratum corneum to a depth of 25 layers. The differences in the effectiveness of the antimicrobial agents must be related to the ability to penetrate into the stratum corneum, since all eight agents killed organisms on the skin surface. Thus, the treatment of skin with an antimicrobial agent can be assumed to be effective in eradicating organisms on the surface but not necessarily in the stratum corneum.

The effect of antimicrobial treatment of the skin surface on the reservoir of the resident flora was examined by determining whether sites at which surface treatment had sterilized the stratum corneum were repopulated after 18 h. At untreated sites, a stable population of resident organisms was maintained under the sterile gauze overnight. Sites treated with iodine, iodophor ointment, and mupirocin ointment were repopulated to the expected level for resident flora (Fig. 3). The surprising finding was that sites treated with the triple-antibiotic ointment were not repopulated. The mechanism by which this ointment, containing neomycin, polymyxin, and bacitracin in a petrolatum base, prevented repopulation of the stratum corneum is not known. The antibiotics may have eradicated the bacteria in the reservoir by penetrating the sebum and traveling down the hair follicle to enter the sebaceous glands in the dermis. Alternatively, the antibiotics may have been bound to tissue in the stratum corneum (4) and may have killed the organisms as they entered the area from the reservoir in the sebaceous glands.

Delineation of the microbial anatomy of normal skin has implications for the care of patients in a clinical setting. In this study, we demonstrated that topical treatment which sterilizes the skin surface usually does not sterilize the underlying stratum corneum and sebaceous glands. An incision through nonsterilized stratum corneum and sebaceous glands is not a problem in most surgical wounds, since the resident CNS have little pathogenic potential. However, contamination of prosthetic material, such as ventriculoperitoneal shunts or artificial heart valves, with the resident CNS from the wound margin during implantation may lead to an infection which necessitates the removal of the implanted device. More commonly, CNS resident in the stratum corneum and sebaceous glands may play a role when a sterile catheter is inserted through the skin into a blood vessel and left in place for several days. The site at which the catheter enters the skin is usually covered with a sterile dressing. In light of the current study, it is reasonable to assume that repopulation of the skin at the entry site occurs within 18 h after sterilization of the surface and insertion of the device. This repopulation occurs regardless of the type of sterile dressing covering the area (1). Pretreatment with a triple-antibiotic ointment may prevent repopulation under these conditions, but even the prevention of repopulation does not prove that patients would benefit by a lower rate of catheter-related infections. The efficacy in the prevention of catheter-related infections of the continuous application of a triple-antibiotic ointment to the catheter entry site throughout the entire time that the catheter was in place was tested (reviewed in reference 7), with inconsistent results. However, it must be remembered that the effect of an ointment is limited to the eradication of organisms on and in treated skin; organisms introduced onto or into a catheter at other sites cannot be blocked by topical skin treatment.

The demonstration that treatment of skin with an ointment containing three antibiotics both eradicated organisms from the stratum corneum and prevented repopulation of the stratum corneum with resident flora provides a tool for further exploration of the behavior of organisms of low pathogenicity.

ACKNOWLEDGMENT

This work was supported by the Pendleton Laboratory Research Endowment Fund, Department of Pediatrics, University of Virginia Health Sciences Center, Charlottesville.

REFERENCES

1. **Aly, R., C. Bayles, and H. Maibach.** 1988. Restriction of bacterial growth under commercial catheter dressings. Am. J. Infect. Control **16**:95–100.
2. **Ayliffe, G. A. J., J. R. Babb, K. Bridges, H. A. Lilly, E. J. L. Lowbury, J. Varney, and M. D. Wilkins.** 1975. Comparison of two methods for assessing the removal of total organisms and pathogens from the skin. J. Hyg. Camb. **75**:259–274.

3. **Brown, B., R. Wenzel, and J. O. Hendley.** 1989. Exploration of the microbial anatomy of normal human skin by using plasmid profiles of coagulase-negative staphylococci: search for the reservoir of resident skin flora. J. Infect. Dis. **160**:644–650.
4. **Kirton, V., and D. Munro-Ashman.** 1965. Contact dermatitis from neomycin and framycetin. Lancet **i**:138–139.
5. **Lovell, D. L.** 1945. Skin bacteria. Their location with reference to skin sterilization. Surg. Gynecol. Obstet. **80**:174–177.
6. **Lowbury, E. J. L., and H. A. Lilly.** 1975. Gloved hand as applicator of antiseptic to operation sites. Lancet **ii**:153–156.
7. **Maki, D. G., and J. D. Band.** 1981. A comparative study of polyantibiotic and iodophor ointments in prevention of vascular catheter-related infection. Am. J. Med. **70**:739–743.
8. **Montes, L. F., and W. H. Wilborn.** 1969. Location of bacterial skin flora. Br. J. Dermatol. **81**(Suppl. 1):23–26.
9. **Pinkus, H.** 1951. Examination of the epidermis by the strip method of removing horny layers. I. Observations of thickness of the horny layer and of mitotic activity after stripping. J. Invest. Dermatol. **16**:383–386.
10. **Selwyn, S., and H. Ellis.** 1972. Skin bacteria and skin disinfection reconsidered. Br. Med. J. **1**:136–140.