Bacteria of Porcine Skin, Xenografts, and Treatment with Neomycin Sulfate

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Homogenized 4-mm punch biopsies were taken from pigs and bacteriologically evaluated to determine the efficacy of surgical scrub procedures and the subsequent treatment of tissue with 0.5% neomycin sulfate-sodium bisulfite (neomycin-bisulfite) as a decontaminating agent. The majority of the lots of porcine skin taken directly from animals for xenografts in the treatment of burns contained viable bacteria at the time of grafting although scrubbing procedures substantially reduced the skin bacteria. The porcine bacteria consisted primarily of coagulase-negative staphylococci with most strains exhibiting caseinolytic and elastase activity. Staphylococci were the only abundant bacteria found in post-scrub biopsies and in saline solutions used to wash the dermatome during its use. After an overnight exposure of grafting tissue soaked in neomycin-bisulfite, the spent neomycin-bisulfite solutions were tested for bacteriostatic and bactericidal activity by comparison to unused neomycin. All solutions tested were equal in bacteriostatic strength, but the bactericidal action of some spent solutions was decreased. Neomycin alone exerted a more lethal effect on sensitive bacteria than the neomycin-bisulfite solution. The desirability of having viable porcine skin for a xenograft necessitated using or discarding the tissue after storage in neomycin-bisulfite at 4°C for a maximum of 72 hr. Certain contaminating microorganisms were unaffected by antibiotic treatment, and the prolonged use of neomycin without bisulfite would have primarily eradicated only the porcine coagulase-negative staphylococci. Neither the presence of this group in grafting tissue nor their proteolytic activity had any observed adverse effect on xenografting success.

Materials and Methods

Xenograft donors. Male or female Yorkshire and Raceland pigs rented from a local farm were maintained in a separate animal laboratory. Pigs usually weighed between 100 and 130 lb (50 and 65 kg) on arrival and reached weights of between 190 and 225 lb (95 and 112 kg) during the donor period. Partial thickness skin grafts (0.008 to 0.010 inch) were removed by a dermatome alternating the sides of each pig. Fifteen to 21 days was allowed for skin regeneration before the same side of the animal was used again. One day prior to graft removal, donors received approximately 10^8 units of procaine penicillin for general surgical prophylaxis. On the day of the operation, the animal was anesthetized with Halothan; the side of the animal to be used was clipped to remove coarse bristles, and additional bristles were removed with a dry razor. The animal was then transported to the operating room.

Surgical scrub procedure and skin removal. Ethyl ether was applied liberally to the shaven side of the animal and rubbed across the surface with...
sterile gauze to remove surface lipids. Concentrated betadine was then added, mixed into a lather with water, and scrubbed over the graft area for 10 min. The scrubbed area was then draped with gauze soaked in Clorox (2 oz/gal of water) for 3 min. When the gauze was removed, sterile mineral oil was applied to the skin to act as a lubricant for the dermolete. Strips of shaved skin were rolled in gauze soaked in saline containing 0.5% neomycin sulfite with 250 mg of sodium bisulfite per liter. The neomycin-bisulfite solutions were prepared in the pharmacy and sterilized by autoclaving. The rolled skin was immersed in the antibiotic in jars and held for 24 hr at 4 C. After this treatment, the tissue was used for grafting to the skin of a patient.

Bacteriological specimens. The coarse topography of porcine skin made surface swabbing unsatisfactory for this study. Efficacy of surgical scrubbing was evaluated by taking full-thickness skin samples with disposable 4-mm skin biopsy punches (Dome Laboratories, West Haven, Conn.) before and after skin disinfection. The specimens were placed in cold saline and examined within 30 min of collection. Each specimen was weighed to the nearest 0.1 mg and cut lengthwise through the tissue plug. The specimen was transferred to a Teflon tissue grinding unit containing 2 ml of leafet broth (BBL). The tubes were kept in crushed ice and homogenized at 9,000 rev/min for 60 sec with a grinding apparatus (Tri-R Instruments, Inc. Rockville Centre, N.Y.). Samples of the homogenate were diluted and plated by methods previously described (8). In some cases, punch biopsy specimens soaked in neomycin-bisulfite for 2 hr were also tested. Saline used to wash the dermolete during its use was collected in sterile disposable containers for enumeration. Sterility tests on the strips of pig skin after removal from the animal and before application to the patient consisted of the following: two cotton wool swabs were rolled over an area of tissue approximately 3 inch² and placed in a tube. A piece of skin weighing 1 to 2 g was cut and placed in a container with saline. The swabs were rinsed in thioglycollate medium (BBL) and streaked on 5% blood-agar, phenylethyl alcohol-blood-agar (BBL), and MacConkey agar (BBL). The swabs were then placed in fresh tubes of thioglycolate. The tissue specimen was homogenized and inoculated into the same media. All media were incubated at 37 C for 72 hr.

Quantitative studies. Total plate counts were made on 5% human blood-agar with Columbia agar base (BBL). Additional media used to aid in identification of bacteria were: KA agar (Difco) for enterococci; ammonium phosphate-agar (BBL) for saprophytic micrococci, mannitol-salt-agar (BBL) for staphylococci, FTO agar (8) for diphtheroids, MacConkey agar (BBL), and Littman oxgall agar (Difco) for yeasts and fungi. The latter medium contained gentamycin (80 µg/ml) in place of streptomycin. Enumeration of proteolytic bacteria was made with the medium of Martley et al. (6).

Incubation. Aerobic plates were incubated at 37 C for 48 hr. Blood plates were also incubated anaerobically for a period of 5 days at 37 C in Brewer jars with GasPaks (BBL) or in Case anaerobic jars flushed three times with a mixture of 95% N₂ and 5% CO₂.

Identification procedures. Members of the Micrococcaceae were identified by the methods of Baird-Parker (1) and Branson (2). Selected strains were tested for elastase activity by the methods of Varadi and Suequeton (9). Diphtheroids and yeasts were identified by Gram stains and wet mounts. Gram-negative bacilli were identified by the methods of Martin (5). Enterococci were identified by growth on KA agar, the catalase test, growth in SF (Difco) and ethyl violet azide broth, growth and hydrolysis on bile esculin agar (BBL), and acid production from 1% broths of mannitol, sorbitol, raffinose, glycerol, arabinose, lactose, and sucrose in phenol red base (BBL).

Antimicrobial assays. Sensitivity to neomycin was determined by the Kirby-Bauer method. Serial twofold dilutions in Trypticase soy broth (BBL) were used for minimal inhibitory concentrations (MIC). Tubes showing no visual growth after 24 hr were streaked on blood-agar and incubated for 48 hr to determine bactericidal activity. Spent neomycin-bisulfite solutions used to decontaminant pig tissue were tested for residual activity by the above methods and by adding these solutions to penicillin cylinders placed on seeded media. Quantitative bactericidal activity was measured by adding cultures to 50-ml screw-cap flasks with 10 ml of test solutions. The flasks were kept at 4 C to simulate the conditions of tissue storage. Samples were removed, diluted, and plated on blood-agar which was incubated for 48 hr.

RESULTS

The efficacy of disinfectant surgical scrubbing was evaluated by enumerating the bacteria of punch skin biopsies of 12 animals before and after treatment (Table 1). Considerable variation occurred in total bacterial counts of each specimen, but a general reduction in skin organisms was observed in each specimen taken after scrubbing. The bacterial species of each specimen before treatment were dominated by coagulase-negative staphylococci which comprised between 70 and 95% of the population. Seventy-eight of these strains were characterized and consisted of mixtures of Staphylococcus epidermidis bio-types II, III, and VI. All of these strains were sensitive to 30-µg discs of neomycin. A group of 31 lactose-fermenting strains was tested for elastolysis, and 22 were positive (Fig. 1). In enumeration studies of biopsies by using the medium of Martley et al. (6), the staphylococci were proteolytic and completely digested casein in 48 hr. The remainder of the bacteria found in each specimen before scrubbing consisted of micrococi, Gaftjhya species, and diphtheroids. Four specimens contained enterococci identified as a mixture of Streptococcus faecalis, S. faecium, and S. bovis. One specimen contained sporeforming aerobic
bacilli. There were no yeasts, gram-negative bacilli, or obligate anaerobes isolated.

In the specimens examined after scrubbing porcine skin, the organisms found were primarily staphylococci. Only one specimen contained Enterobacter aerogenes which accounted for 5% of the population in that specimen. Saline used to wash the dermatome during its use contained bacteria ranging from 20 to 3,100 per ml with an average of 650 per ml. One solution contained Escherichia coli, but S. epidermidis was the only other species isolated and was found in every saline wash solution.

A sequential examination of biopsies including tests on strips of skin placed in neomycin-bisulfite for 2 hr and 24 hr at 4 C demonstrated that the eradication of bacteria from the skin was dependent on the initial number of bacteria present at the time of graft removal (Table 2).

During the time in which biopsies were examined, 114 sterility tests on freshly removed skin and skin soaked in neomycin-bisulfite resulted in 81 (71%) of the specimens being positive (Table 3). Swabbings and tissue strips were equally positive for each animal and specimens positive before neomycin-bisulfite treatment were also positive after 24 hr of antibiotic treatment. The majority of the organisms were coagulase-negative staphylococci, but 21% of the organisms were gram-negative. The large number of positive tissue specimens occurred despite the antimicrobial activity of used neomycin-bisulfite solutions which frequently contained blood, fat, and other tissue debris (Table 4).

Experiments were then conducted which showed that the sodium bisulfite in a concentration of 250 mg/liter had no effect on bacteria. When the MIC values of neomycin alone and neomycin-bisulfite preparations were compared on strains of S. epidermidis, the bacteriostatic properties of the solutions were equal. The bactericidal activity of neomycin, however, was substantially reduced by the addition of bisulfite to the neomycin (Fig. 2). Identical results were obtained with three additional strains of S. epidermidis and two strains of Staphylococcus aureus. When the viability of each strain was determined over a period of 24 hr at 4 C, one strain of S. faecalis was unaffected by neomycin or neomycin-bisulfite. In testing one strain of E. coli, neo-

### Table 1. Efficacy of surgical scrubbing in the disinfection of porcine skin

| Animal | Total aerobic count (bacteria/mg)*<br>Before scrub | After scrub |
|--------|---------------------------------------------------|-------------|
| 48     | 4,300                                             | 227         |
| 51     | 1,758                                             | 1,104       |
| 50     | 3,190                                             | 283         |
| 41     | 1,704                                             | <1          |
| 51     | 465                                               | 16          |
| 54     | 2,160                                             | <1          |
| 55     | 3,143                                             | 102         |
| 54     | 410                                               | 8           |
| 51     | 13,200                                            | 4,114       |
| 52     | 105                                               | 17          |
| 53     | 425                                               | 288         |
| 57     | 67                                                | 16          |
| 56     | 237                                               | 40          |
| 57     | 70                                                | 6           |
| 58     | 111                                               | 2           |
| 43     | 693                                               | 2           |

* Order of specimens was by weekly sequence of animals used in some cases alternating right and left sides.

* Average weight of biopsies was 38 mg.

![Fig. 1. Elastase activity of three of four porcine strains of Staphylococcus epidermidis. Plates were incubated in candle jar at 37 C for 48 hr. Medium contained Trypticase soy agar with 1% elastin powder. Plate on right shows elastin degradation of same strain as on left plate after growth was removed with a swab.](http://aem.asm.org/)

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TABLE 2. Comparison of tissue disinfection resulting from surgical scrubbing and subsequent decontamination by neomycin-bisulfite

| Sample                        | Bacterial count in sample from animal donor* |
|-------------------------------|---------------------------------------------|
| Biopsy before scrub           | 2,956 536 6                                |
| Biopsy after scrub            | 213 254 <1                                 |
| Dermatome rinse water         | 1,040 160 10                               |
| Tissue in neomycin 2 hr       | 110 12 <1                                  |
| Tissue before application to patient (in neomycin 24 hr) | 80 2 <1 |

* All total bacterial counts expressed per milligram except from dermatome rinse water which is expressed per milliliter.

TABLE 3. Microorganisms isolated from freshly stripped and neomycin-bisulfite-soaked porcine skin*

| Group         | No. of specimens containing microorganisms* | Organisms identified                  | Sensitivity in 30-μg neomycin disc* |
|---------------|---------------------------------------------|---------------------------------------|-------------------------------------|
| Gram-positive | 76                                          | Staphylococcus epidermidis            | S                                   |
|               | 5                                           | enterococci                           | R                                   |
|               | 2                                           | Staphylococcus aureus                 | I                                   |
|               | 1                                           | Candida sp.                           | R                                   |
| Gram-negative | 4                                           | Escherichia coli                      | S, 3I                               |
|               | 3                                           | Klebsiella pneumoniae                 | I                                   |
|               | 2                                           | Proteus sp.                           | S                                   |
|               | 1                                           | Pseudomonas sp.                       | R                                   |

* Freshly stripped skin as removed directly from pig. Treated tissue was placed in antibiotic solution for 24 hr at 4 C.

** Tests conducted with a strain of Staphylococcus epidermidis.

*** Number refers to animal donor tissue on which each solution was used.

mycin-bisulfite decreased the viability of the strain after 24 hr by 32%, whereas neomycin alone caused a 70% reduction.

It was noted that autoclave-sterilized neomycin sulfate developed a brownish color, but the addition of 250 mg of sodium bisulfite per liter prevented the discoloration of neomycin. Filter-sterilized neomycin and neomycin-sulfite both remained colorless. When the bacterial activity of the filtered and autoclaved solutions were compared, the sodium bisulfite continued to reduce the lethal effects of the neomycin.

DISCUSSION

The results of this study illustrate several paradoxical situations concerning porcine xen-
attempts there skin thrives useful adversely difficulties involved in ridding bacteria from the pig skin. The predominance of the coagulase-negative staphylococci on the skin before scrubbing and their nearly exclusive occurrence in skin after scrubbing and in dermato-mere wash water indicate that this group thrives at some depth in the pig skin unaffected by surgical scrubs. This could include sebaceous units or skin follicles, as this occurs in humans (7). Even though this group of bacteria is sensitive to neomycin, their numbers in the skin and the great surface area of stripped skin soaked in neomycin makes the elimination of this group difficult. Since gram-negative bacilli were not isolated from pig biopsies, these organisms cannot be considered resident skin organisms. Their recovery from stripped skin may be the result of fecal contamination, but their resistance to neomycin as with the enterococci means that neither group would be eradicated from the skin for grafting procedures by antibiotic treatment.

The deletion of bisulfite from neomycin and treating the tissue for longer periods would increase the antibacterial activity of neomycin and further eradicate staphylococci from the skin. The porcine tissue which is viable is most useful when applied to the patient 24 hr after removal from the animal. It cannot be kept more than 72 hr before it is discarded. The complete eradication of all bacteria from the skin by antibiotics may also be undesirable. Over the past 4 years of porcine xenografting, there has been no evidence that staphylococci adversely affect the grafting operation which attempts to hold porcine skin on the granulating bed of the patient for a minimum of 5 days. Within 4 hr after porcine skin is applied, swabblings from under the graft revealed proliferation of the staphylococci (unpublished data). The role of staphylococcal proteolysis, including elastase activity, requires further study. Finally, the staphylococci may, by their presence in skin, prevent other more potentially harmful organisms from colonizing the graft. This possibility is also being studied.

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