Comparative Characteristics of Human and Porcine Staphylococci and Their Differentiation in Burn Xenografting Procedures

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Staphylococcus epidermidis from porcine skin differed from human cutaneous S. epidermidis in that the former strains were principally of the Baird-Parker biotype III group. The porcine-type strains were more proteolytic on casein and gelatin than were human strains, which were primarily of biotype II. Porcine strains were also elastolytic. Using supernatant fluids of broth cultures, the biotype II strains, but not the type III strains, were lipolytic in action on triolein. Both types of staphylococci were similar in enzymatic activities on Tween 80, egg yolk, and tributyrin. Elastase activity was not found in broth supernatant fluid of these bacteria. The porcine strains were retarded or inhibited from growing in media at pH 5.5. Action on casein agar followed by demonstration of elastase activity were used as markers to detect the porcine S. epidermidis strains in xenografts and on human burn wound grafting sites.

Several studies have been focused on the biochemical differences between strains of Staphylococcus aureus from human and animal sources. Elek and Levy (5) found that human strains generally produced alpha hemolysin whereas animal strains produced beta hemolysin. Marandon and Oeding (6) compared human and bovine strains of S. aureus by using several biochemical tests and found beta hemolysin production and the inability to produce fibrinolysin to be valid criteria of bovine strains.

Baird-Parker (1), in devising a scheme for biotyping members of the family Micrococceae, reported that Staphylococcus epidermidis subgroups II and III predominated on pig skin whereas S. epidermidis subgroup III was not isolated from human skin. Smith and Evans (9) examined the bacterial flora of porcine skin prepared as xenografts for application to burns of children and reported that coagulase-negative staphylococci from porcine donors exhibited elastase activity and were caseinolytic. This study deals with further comparative characteristics of cutaneous staphylococci of human and porcine origin.

MATERIALS AND METHODS

Source of strains. Porcine staphylococci were derived from surgically removed porcine skin used for xenografts by the methods described by Smith and Evans (9). Human strains of staphylococci were obtained from skin swabblings taken randomly from adults and children and plated on various media by using the methods of Smith (8).

Comparative characteristics of staphylococci. Populations of staphylococci from human and porcine sources were diluted and plated on several media. This was not done for enumeration per se, but to compare and measure the activities of similar numbers of colonies of the two populations of staphylococci on several substrates. Total plate counts were made with 5% human blood agar (Columbia agar base, BBL), and manniot salt agar (BBL) was used to determine the approximate numbers of staphylococci in each sample. Casein hydrolysis was determined with the medium of Martley et al. (7). Gelatinase activity was detected by flooding plates of nutrient agar (Difco) containing 0.4% gelatin with acidic HCl. The egg yolk reaction was measured on Trypticase soy agar (BBL) containing a final concentration of 5% egg emulsion and 1 g of CaCl2 per liter. Hydrolysis of Tween 80 was detected on nutrient agar (Difco) containing 10 g of Tween 80 and 1 g of CaCl2 per liter. Lipase action was detected on Spirit blue agar with tributyrin (Difco) and with Victoria blue triolein agar. Triolein (Sigma Chemical Co., St. Louis, Mo.) was prepared as a 10% emulsion in 10% aqueous gum acacia. The emulsion was stabilized with a Branson sonifier (model 140 D) at one-half maximum power for 3 min with the emulsion packed in crushed ice. A few drops of chloroform were added as a preservative. The emulsion was stored at 4 C and...
discarded after 1 week. A final concentration of 0.1% emulsion was added to sterilized Trypticase soy agar without glucose (BBL) supplemented with 0.1% yeast extract and 0.0015% Victoria blue. The final pH of this medium was 5.5 adjusted with concentrated HCl and tested potentiometrically. All of the above media were incubated aerobically at 34 C for 48 hr. Total colony counts were made on each medium, and plates containing comparable numbers of staphylococci of porcine and human origin were compared for their activities on casein, gelatin, Tween 80, egg yolk, tributyrin, and triolein.

Identification and tests on pure cultures of staphylococci. Strains representative of the staphylococci from the two sources were identified and biotyped by the Baird-Parker classification scheme (2). Elastase activity was determined by the methods of Varadi and Saqueton (11) by using purified elastin powder (Sigma). Constitutive enzyme production of strains was compared by growing the organisms in brain heart infusion broth (BBL) overnight at 34 C to a concentration of approximately 5 x 10^8 viable cells per ml. The broths were centrifuged at 5,000 rev/min for 10 min, the supernatant fluids were removed, and a few drops of chloroform were added to each sample. This procedure effectively sterilized the broth. Holes were cut into several types of media with a 10-mm corkborer, and 0.2-ml quantities of the broth supernatant fluids were added to the wells. A set of broths, which included one portion boiled for 25 min as a control, was placed in the incubator at 34 C, and zones of enzyme activity were recorded. Growth at pH 5.5 was determined by inoculating Tryptase soy broth and streaking Tryptase soy agar adjusted to pH 5.5. The same medium at pH 7.2 was inoculated as a control. The results of these tests were recorded after 48 hr of incubation.

Microbiology of skin grafting sites. Porcine xenografts usually contain staphylococci at the time the tissues are applied to patients (9). Information derived from this study was utilized to attempt to identify and differentiate porcine and human staphylococci on grafting sites and grafts after application and removal or rejection of the graft. Microorganisms present at the grafting site prior to xenografting were determined by using the gauze capillary culture method (4). The xenograft microflora and any organisms found on rejected or discarded xenografts were identified by the methods of Smith and Evans (9). In addition to the normal battery of media used to isolate and identify organisms in these specimens, casein agar was added to presumptively identify porcine staphylococci. Isolates considered to be of porcine origin were then transferred in elastin agar to detect elastase activity.

RESULTS

The relative biochemical activities of S. epidermidis from 12 porcine xenografts and 12 swablings of normal human adult skin were compared on six substrates (Table 1). Mannitol salt agar was used to presumptively identify S. aureus in specimens. Specimens which contained this organism after confirmation by the tube coagulase test were discarded. Swablings from normal adult skin were used because the majority of the acutely burned children were colonized by an endemic strain of S. aureus. The porcine staphylococci were uniformly more active in proteolysis against casein and gelatin than were human populations of coagulase-negative strains. Lipolytic and lecinthinase activity in the two groups of staphylococci were variable and moderate when positive. The porcine staphylococci, however, grew poorly or not at all on the triolein Victoria blue agar. This medium was adjusted to pH 5.5 which is the usual pH of normal human skin. Triolein was used as a substrate because it is a major component of human sebum. Furthermore, zones of hydrolysis against triolein were more demonstrable at pH 5.5 than at pH 7.0. Twelve strains of porcine and human staphylococci were tested for growth at pH 5.5 and 7.0 in Tryptase soy broth and on the same agar medium. The human strains grew equally at both pH levels, but the porcine strains were inhibited from growing at pH 5.5.

To compare more fully the relative biochemical activities of these bacteria, constitutive enzyme production was measured from broth supernatant fluids of representative strains (Table 2). The predominant staphylococci from porcine xenografts were Baird-Parker biotype III, and those of normal human skin were biotype II. The two groups of strains had similar degrees of activity against tributyrin, egg yolk, and Tween 80, but only the biotype II strains exhibited activity on triolein. The porcine strains were stronger in proteolytic action on

| Medium activity          | Source and activity of staphylococci |
|--------------------------|--------------------------------------|
|                          | Porcine xenografts | Normal human skin |
| Caseinolysis             | 3-4+                 | 0-2+               |
| Gelatinase               | 3-4+                 | 1-2+               |
| Tween 80 hydrolysis      | 0-1+                 | 0-1+               |
| Triolein hydrolysis      | 0-1+                 | 0-1+               |
| Tributyrin hydrolysis    | 0-1+                 | 0-1+               |
| Egg yolk lecinthinase    | 0                    | 0                   |

* Based on comparative activity of similar numbers of isolated colonies of populations of coagulase-negative staphylococci enumerated on various media for 48 hr.

* Enzyme activity was scored in relative terms of strength judged from diameters of zones around colonies: 0, negative; 1-4+, positive.
A detailed interpretation of the document is as follows:

- The document discusses the enzymatic activities of Staphylococcus aureus, focusing on caseinolysis, elastase, and gelatinase.
- A table compares the activity of broth supernatant fluids of porcine and human Staphylococcus epidermidis.
- The table indicates that porcine strains had a higher range of zone size compared to human strains.
- There was a notable activity of porcine and human strains in terms of caseinolysis, elastase, and gelatinase.
- The discussion section elaborates on the characteristics of porcine and human strains, indicating that porcine strains were more active in terms of caseinolysis, elastase, and gelatinase.
- The text mentions the utilization of agar wells containing broth supernatant fluid and the incubation of cultures.
- The study indicates that porcine strains are more active in terms of enzymatic activities compared to human strains.
- The discussion section emphasizes the importance of these findings in the context of wound care and the potential implications for treatment.

The document also includes a table comparing the elastase activity of staphylococci from human and porcine sources, highlighting the variability in the number of strains positive for total tested between porcine and human skin.

In conclusion, the document provides valuable insights into the enzymatic activities of Staphylococcus aureus and their implications for wound care and treatment.
differed also in their lipolytic activity compared to the biotype II and other strains tested.

Varadi and Saqueton (10) observed that S. epidermidis, Baird-Parker type I, referring to a later designation given to biotype II coagulase-negative staphylococci (3), reported that this type of staphylococcus was implicated in the etiology of human cutaneous perifollicular elastolysis. It was also found that bacteria-free extracts of culture media on which an elastase-negative strain of S. epidermidis was grown were elastolytic on human skin (11). This would imply that the elastase was constitutively produced by the human strain. It has not been determined whether elastase of porcine staphylococci is produced in vivo and has any role in burn xenografting procedures. It is of interest, however, that the separation of burn eschar is closely related to dissolution of elastic tissues (12).

The use of casein and elastin agars to detect the presence of porcine biotype III staphylococci on human skin following application of porcine xenografts to the patients appears to have promise as a presumptive rapid means of distinguishing these strains from human types. The detection of the biotype III staphylococci in only one of six rejected or discarded grafts could be accounted for by one of two reasons. Either the staphylococci generally do not colonize the burn sites, or they are overwhelmed and obscured from detection when other thermocutaneous pathogens are present. A series of selective agents are now being studied to attempt to incorporate an inhibitor in casein agar selective for the staphylococci. This might aid in revealing the presence of the porcine strains in the specimens containing other organisms.

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