APPLIED MICROBIOLOGY, Dec. 1972, p. 929-932
Vol. 24, No. 6

Printed in U.S.A.

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

RODNEY F. SMITH AND CAROL L. BETTGE

Division of Microbiology, Shriners Burns Institute, University of Texas Medical Branch, Galveston, Texas 77550

Received for publication 21 August 1972

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 *p*H 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 *Micrococcaceae*, 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 swabbings 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 mannitol 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 $HgCl_2$. The egg yolk reaction was measured on Trypticase soy agar (BBL) containing a final concentration of 5% egg emulsion and 1 g of $CaCl_2$ per liter. Hydrolysis of Tween 80 was detected on nutrient agar (Difco) containing 10 g of Tween 80 and 1 g of $CaCl_2$ 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 *p*H 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 5×10^8 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 *p*H 5.5 was determined by inoculating Trypticase soy broth and streaking Trypticase soy agar adjusted to *p*H 5.5. The same medium at *p*H 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 swabbings 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. Swabbings 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 lecithinase 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 *p*H 5.5 which is the usual *p*H 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 *p*H 5.5 than at *p*H 7.0. Twelve strains of porcine and human staphylococci were tested for growth at *p*H 5.5 and 7.0 in Trypticase soy broth and on the same agar medium. The human strains grew equally at both *p*H levels, but the porcine strains were inhibited from growing at *p*H 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

TABLE 1. *Enzyme activity of coagulase-negative porcine and human staphylococci*[a]

Medium activity	Source and activity of staphylococci[b]	
	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 lecithinase	0	0-1+

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

[b] Enzyme activity was scored in relative terms of strength judged from diameters of zones around colonies: 0, negative; 1-4+, positive.

TABLE 2. *Comparative enzyme activity of broth supernatant fluids of porcine and human Staphylococcus epidermidis*[a]

Enzyme activity	Porcine group III			Human group II		
	Strains[b]	Range of zone size	Average zone size	Strains	Range of zone size	Average zone size
Caseinolysis	21/21	14-30	25	13/20	13-20	15
Gelatinase	21/21	24-35	30	18/20	15-20	17
Elastase	0/21	0	0	0/20	0	0
Triolein	0/21	0	0	20/20	14-19	17
Tributyrin	14/21	14-25	17	19/20	14-24	20
Lecithinase	5/21	12-14	13	14/21	13-16	14
Tween 80	4/21	20-23	22	19/20	16-19	18

[a] Agar wells with 10-mm diameter containing 0.2 ml of broth supernatant fluid were incubated at 37 C for 24 hr. Diameter of zones measured in millimeters for comparative enzymatic activity.

[b] Number of strains positive/number of strains tested.

gelatin and casein. There was no elastase activity found in any of the supernatant fluids.

The elastase activity of staphylococci from porcine xenografts and from swabbings of patients were then compared (Table 3). The large number of biotype I (*S. aureus*) strains found on children was due to the excessive colonization of burns and normal skin of the patients by the *S. aureus*. The five strains of biotype III isolated from two of 12 acute patients were considered to be the result of these patients having been previously xenografted. Biotype III strains were not found on skin or burns of the other 10 acute patients who at the time of culturing had not received porcine xenografts. The biotype III strains were the only strains of staphylococci which were elastase positive.

The strong caseinolytic and elastase activities of the porcine biotype III strains were utilized as possible markers to presumptively differentiate and enumerate these bacteria in rejected or discarded xenografts applied to patients (Table 4). Six porcine tissues containing from 10^2 to 10^3 biotype III staphylococci per g were applied to burn sites of six individuals who had variable numbers of *S. aureus* and *Pseudomonas aeruginosa* on the grafting beds. After the removal of the six grafts, which varied both in time and the circumstances concerned with graft removal, the biotype III strains from the xenograft used were isolated from one discarded graft after it had been on the patient for 6 days. In this instance, the staphylococcal colonies on casein and blood agars were readily separable from those of the pseudomonad, but the marked caseinolytic action of the staphylococci was suggestive of the biotype III strains, and subculture of these types to elastin agar demonstrated the elastase action of these types. Baird-Parker typing also confirmed these strains as type III.

TABLE 3. *Elastase activity of staphylococci from human and porcine sources*

Baird-Parker group	No. of strains positive of total tested[a]	
	Porcine xenografts	Human skin & burns
I	—[b]	0/55
II	0/2	0/28
III	38/38	5/5
IV	—	0/1

[a] Strains were picked randomly from total plate counts of 12 xenografts and 12 swabbings of normal skin and burns of children. Elastin plates were incubated for 48 hr in candle jars.

[b] Not detected.

DISCUSSION

Baird-Parker (1) described 24 strains of biotype III staphylococci which had caseinolytic and gelatinase activity, cleared egg yolk, and were lipolytic on lower Tweens. In the same study, the majority of a much larger group of biotype II staphylococci had the same types of biochemical activity. Baird-Parker also determined in another study that biotype III strains were found on porcine skin and not human skin (2). This study has confirmed the above findings concerning the activity of biotype III staphylococci and their distribution and prevalence in human and porcine skin. The results of this study showed further that the porcine biotype III strains possessed features other than the minimum determinative characteristics routinely used to biotype staphylococci. These features included greater constitutive proteolytic activity on casein and gelatin than biotype II strains of human or porcine origin and elastase activity which appeared to be an induced enzyme. The porcine strains were inhibited or retarded from growing at *p*H 5.5 and

TABLE 4. *Bacteria present at burn grafting sites and on discarded or rejected xenografts*[a]

Sample graft site	Bacteria per cm^2 before graft	Bacteria per g graft removed	Time graft on patient	Clinical evaluation of removed graft
1. Arm	300 *P. aeruginosa*	8×10^6 *P. aeruginosa*	48 hr	Removed-purulent
2. Thigh	600 *P. aeruginosa*	3×10^5 *P. aeruginosa* 1×10^5 *S. epidermidis* biotype III	6 days	Successful
3. Back	1.6×10^5 *S. aureus*	6×10^5 *S. aureus*	24 hr	Removed-purulent
4. Chest	<25	2.6×10^2 *S. aureus*	6 days	Successful
5. Arm	<25	1×10^7 *S. aureus* 1×10^8 *P. aeruginosa*	5 days	Removed-purulent
6. Back	120 *S. aureus*	1.36×10^5 *S. aureus*	24 hr	Satisfactory at time, removed for other surgery
	400 *P. aeruginosa*	1.06×10^3 *P. aeruginosa*		

[a] Each set of graft sites and xenografts was taken from a different patient.

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 1, 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-positive 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.

ACKNOWLEDGMENTS

This work was supported by funds from the Shriners of North America and the McLaughlin Faculty Fund, University of Texas Medical Branch, Galveston.

We thank Wesley E. Kloos for his helpful suggestions in the preparation of this manuscript.

LITERATURE CITED

1. Baird-Parker, A. C. 1962. The occurrence and enumeration, according to a new classification, of micrococci and staphylococci in bacon and on human and pig skin. J. Appl. Bacteriol. **25**:352-361.
2. Baird-Parker, A.C. 1963. A classification of micrococci and staphylococci based on physiological and biochemical tests. J. Gen. Microbiol. **30**:409-427.
3. Baird-Parker, A. C. 1965. Staphylococci and their classification. Ann. N.Y. Acad. Sci. **128**:4-25.
4. Brentano, L., C. A. Moyer, D. L. Gravens, and W. W. Monafo, Jr. 1966. Bacteriology of large human burns treated with silver nitrate. Arch. Surg. **93**:456-466.
5. Elek, S. D., and E. Levy. 1950. Distribution of haemolysins in pathogenic and nonpathogenic staphylococci. J. Pathol. Bacteriol. **62**:541-554.
6. Marandon, J. L., and P. Oeding. 1966. Investigations on animal Staphylococcus aureus strains. 1. Biochemical characteristics and phage typing. Acta Pathol. Microbiol. Scand. **67**:149-156.
7. Martley, F. G., S. R. Jayashankar, and R. C. Lawrence. 1970. An improved medium for the detection of proteolytic organisms in total bacterial counts. J. Appl. Bacteriol. **33**:363-370.
8. Smith, R. F. 1970. Comparative enumeration of lipophilic and nonlipophilic cutaneous diphtheroids and cocci. Appl. Microbiol. **19**:254-258.
9. Smith, R. F., and B. E. Evans. 1972. Bacteria of porcine skin, xenografts, and treatment with neomycin sulfate. Appl. Microbiol. **23**:293-297.
10. Varadi, D. P., and A. C. Saqueton. 1968. Elastase from *Staphylococcus epidermidis*. Nature (London) **218**:468-470.
11. Varadi, D. P., and A. C. Saqueton. 1970. Perifollicular elastolysis. Brit. J. Dermatol. **83**:143-150.
12. Vistnes, L. M., and G. R. Hogg. 1971. The burn eschar—a histopathological study. **48**:56-60.