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Comparison of Rabbit and Pig Plasma in the Tube Coagulase Test

J. W. YRIOS

Wisconsin State Laboratory of Hygiene, Madison, Wisconsin 53706

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A total of 627 clinical isolates of *Micrococcaceae* were characterized according to their ability to coagulate rabbit and pig plasma, produce thermostable nuclease, and anaerobically ferment glucose and mannitol. By using these characteristics, 416 of the isolates were classified as *Staphylococcus aureus* and 211 as non-*S. aureus*. All 416 strains produced a 3+ to 4+ clot formation in heparinized pig plasma, whereas 415 isolates produced a similar reaction in citrated rabbit plasma. All of the *S. aureus* strains possessed a thermostable nuclease, whereas only four of the non-*S. aureus* isolates exhibited this characteristic. The results obtained using heparinized pig plasma were almost identical to those obtained with commercial rabbit plasma.

Commercially prepared rabbit plasma is routinely used in performing the coagulase test for *Staphylococcus aureus* in most microbiological laboratories. Orth et al. (4), in a comparison of several animal plasmas, concluded that heparinized pig plasma was superior to the other sera tested for use in the plate test for coagulase production. The usefulness of pig plasma in the pour plate method for the detection of coagulase production was later confirmed by Parisi et al. (5). A limited amount of data is available in the literature for comparing the performance of pig plasma in the tube coagulase test for clinical isolates of *S. aureus* (K. F. Weiss, R. N. Robbins, and M. S. Bergdoll, Abstr. Annu. Meet. Am. Soc. Microbiol., 1972, E135, p. 23).

This study was initiated to determine if pig plasma was as effective as rabbit plasma in performing the tube coagulase test, using recent clinical isolates of *Micrococcaceae*. To further characterize the isolates, a thermostable nuclease test and anaerobic fermentation of the glucose and mannitol were performed.

MATERIALS AND METHODS

Bacterial strains. A total of 627 clinical bacterial isolates were collected from a variety of routine specimens submitted to the General Bacteriology Section, Wisconsin State Laboratory of Hygiene, Madison, Wis. All isolates were initially characterized as gram-positive, catalase-positive cocci. The organisms were isolated on sheep blood agar plates, transferred to brain heart infusion broth (Difco), and frozen at −70°C until needed.

Coagulase plasmas. Dehydrated rabbit plasmas (ethylenediaminetetraacetate [EDTA] and citrate) were purchased commercially (Difco) and used according to the manufacturer's instructions. The pig blood was collected at the time of slaughter at the Meat and Animal Science Department, University of Wisconsin, Madison, and pooled for use. Approximately 450 ml of blood was allowed to flow into a 1,000-ml plastic screw-cap bottle containing one of the following anticoagulant solutions: (i) 50 ml of 0.85% NaCl containing 0.5 g of disodium EDTA (Fischer Scientific Co., Fairlawn, N.J.); (ii) 50 ml of 0.85% NaCl containing 5,000 U of sodium heparin (Fischer Scientific Co.). After collection, the blood contained either 0.1 g of EDTA per ml or 10 U of heparin per ml. The blood was immediately centrifuged for 15 min to remove the cellular debris, "rough" filtered in a Seitz filter, and filter-sterilized using a membrane filter (0.22 μm; Millipore Corp.). Samples (11 ml) of the filtrate were placed into screw-cap tubes and frozen (−20°C) until needed.

Coagulase test. Approximately 0.1 ml of an overnight culture of each test strain in brain heart infusion broth (3 ml) was inoculated into a tube containing 0.5 ml of the appropriate plasma. The tubes were incubated at 35 to 37°C and observed for clot formation at 2, 4, and 24 h. The degree of clot formation was rated 1+ through 4+, according to the reactions of Turner and Schwartz (9). A 1+ was small, unorganized (threadlike) clots; a 2+ consisted of a small, organized clot; a 3+ was represented by a large, well-formed clot (moves when tube is inverted); and a 4+ was denoted by a firm clot, which did not move upon inversion of the tube.

Thermostable nuclease. The toluidine blue O-deoxyribonucleic acid agar of Lachica et al. (2) was used. Approximately 15 to 20 ml of the agar mixture was poured into a plastic petri dish (15 by 150 mm). After cooling the agar, 10 equally spaced 3-mm wells were cut into the agar, using a suction device. The overnight brain heart infusion broth culture of each test organism was placed into a boiling-water bath for 15 min and allowed to cool. Each of the wells was filled with one of the previously boiled broth cultures, using a disposable Pasteur pipette. The plates

were incubated at 35 to 37°C and observed at 1, 4, and 24 h for the formation of a typical pink halo around each test well, indicating the presence of a heat-stable deoxyribonuclease.

Carbohydrate fermentations. The ability of the organisms to utilize glucose and mannitol was determined using the media and procedures proposed by the Subcommittee on Taxonomy of Staphylococci and Micrococci (8). The inoculated tubes were incubated in GasPak jars (BBL), and results were recorded after 5 days at 35 to 37°C.

RESULTS

The comparison of results obtained with the 627 isolates of *Micrococcaceae* in the coagulase plasmas and other biochemical characteristics are presented in Table 1. An organism was classified as *S. aureus* if it had the ability to produce a 3+ to 4+ clot in either plasma and possessed a thermostable nuclease. The non-*S. aureus* category consisted of strains that did not have this dual ability. The latter group includes *S. epidermidis* and the micrococci.

A total of 416 of the 627 isolates tested are grouped as *S. aureus*. All 416 of these organisms possessed a thermostable nuclease along with the ability to coagulate each plasma tested. The close correlation between thermostable nuclease and coagulase production further substantiates the findings of previous investigators (1, 3, 6, 7). Seventeen of the 416 strains were unable to ferment mannitol anaerobically.

Clot formation ability of the bacterial strains in the four plasmas is depicted in Table 2. The formation of a firm clot occurred in 406, 415, 412, and 348 isolates using rabbit-EDTA, rabbit-citrate, pig-heparin, and pig-EDTA plasmas, respectively. A 3+ clot was judged as one that was well formed but would move when the tube was inverted. A total of 64 isolates produced this type of clot using pig-EDTA plasma, as compared with 8, 0, and 4 isolates using rabbit-EDTA, rabbit-citrate, and pig-heparin plasmas, respectively. Very few organisms formed a 1+ to 2+ reaction in the plasmas tested. All clots produced by *S. aureus* (416 isolates) were forming by 2 h and had completed formation by 4 h in all the plasmas. The only result obtained with further incubation (24 h) was that six isolates tested in the citrated rabbit plasma reverted from a 4+ to a 2+ by the end of the incubation period. Another observation occurred in this study through technician error, when several enterococci were inoculated into the plasmas. These enterococci all produced a delayed weak clot (false positive) after the 4-h observation time (approximately at 6 h) in the citrated rabbit plasma. Also depicted in Table 1 are the reactions of the non-*S. aureus* strains. A total of 113 organisms exhibited a pattern that might be described as "typical" *S. epidermidis* reactions. Eighty-four of the 211 organisms in this group did not react in any of the tests used, so they probably fall in the micrococci group. The nine organisms that demonstrated coagulase ability in the non-*S. aureus* group did so only in the rabbit-EDTA plasma. The clot reactions noted were 1+ to 2+, which would be of questionable significance according to some recent work done on interpretation of degree of clot formation in the tube coagulase test (6, 7). The nine strains probably represented eight *S. epidermidis* and one micrococcus. Only one non-*S. aureus* isolate anaerobically fermented mannitol and four non-*S. aureus* isolates produced a thermostable nu-

TABLE 2. *Degree of clot formation among the various plasmas*

Plasma	No. of strains with clot formation of:			
	1+	2+	3+	4+
Rabbit-EDTA	0	2	8	406
Rabbit-citrate	0	1	0	415
Pig-heparin	0	0	4	412
Pig-EDTA	1	3	64	348

TABLE 1. *Comparison of coagulase plasma and biochemical characteristics of the bacterial strains*

Biochemical characteristic	Identification of strains							
	S. aureus		Non-*S. aureus*					
	399[a]	17	113[a]	84	8	4	1	1
Rabbit plasma-EDTA	+	+	−	−	+	−	−	+
Rabbit plasma-citrate	+	+	−	−	−	−	−	−
Pig plasma-heparin	+	+	−	−	−	−	−	−
Pig plasma-EDTA	+	+	−	−	−	−	−	−
Thermostable nuclease	+	+	−	−	−	+	−	−
Glucose (anaerobic)	+	+	+	−	+	+	+	−
Mannitol (anaerobic)	+	−	−	−	−	−	+	−

[a] Number of strains.

clease. These latter five isolates were found to be negative in their ability to produce enterotoxin, thus reducing the likelihood that they were enterotoxin-producing strains of *S. aureus* that lost their ability to produce coagulase (3). They most likely represent atypical strains of *S. epidermidis*. A definite identification of these isolates was not performed in this study. Future studies should include tests (cell wall structure, protein A, and phage susceptibility) that would definitely establish the identity of these questionable non-*S. aureus* strains.

DISCUSSION

The ability of clinical isolates of *S. aureus* to produce clots in pig plasma and commercial rabbit plasma appears to be equal. Sperber and Tatini (7) and Rayman et al. (6) disagree slightly on the degree of clotting that should be considered as positive evidence of coagulase production. Our results and observations tend to support the conclusion of Rayman et al., in that a 3+ or 4+ level of clot formation is indicative of *S. aureus*. Below that level in this study, a total of 16 isolates produced 1+ to 2+ reactions. Nine of the 16 strains produced a clot only in rabbit-EDTA plasma and did not appear to be *S. aureus* considering the other biochemical test results. However, the other seven strains were grouped as *S. aureus*, taking into account all the characteristics tested. Six of these seven strains exhibited this weak clot-forming ability in the EDTA plasmas (rabbit and pig). It appears that a thermostable nuclease test done on these "questionable" coagulase-positive strains (1+ and 2+) would correctly categorize them as either *S. aureus* or non-*S. aureus* strains.

One isolate, upon repeated testing, demonstrated a 4+ reaction in heparinized pig plasma while only producing a 2+ in rabbit plasma (EDTA and citrate). This represents the only instance when the heparinized pig plasma varied by more than one clotting level from the commercial rabbit plasma. Therefore, the coagulase results obtained using either plasma were basically identical.

Lot-to-lot variation in rabbit and pig plasmas could theoretically influence the results. However, no observable variation occurred in this study using three lots of commercial rabbit plasma and three lots of pig plasma. Of the lots of commercial rabbit plasma tested, it appears that citrated rabbit plasma exhibited the best degree of clot formation. As expected, problems were encountered using the latter plasma in the form of delayed false positives involving members of the enterococci group and dissolving of the clot with extended incubation (24 h). Therefore, it was observed that an extended incubation period beyond 4 h was not useful.

The heparinized pig plasma appeared to be superior to the pig-EDTA plasma, because most of the isolates produced a well-formed 4+ clot in the former plasma.

Barry et al. (1) suggested the routine use of both the coagulase and thermostable nuclease tests for the routine identification of *S. aureus*. The thermostable nuclease test can be simply performed in conjunction with the tube coagulase test, using the same brain heart infusion broth and an agar that is easily prepared and stored. As shown here, the strains that produced questionable coagulase reactions (1+ and 2+) could be identified as *S. aureus* or non-*S. aureus* on the basis of their ability to produce thermostable nuclease.

Orth et al. (4) proposed that pig plasma is superior to rabbit plasma for performing the plate coagulase test for *S. aureus*. The probable reason for the superiority of pig plasma over that of rabbit plasma is that the former generally has greater amounts of coagulase reacting factor and, at the same time, a lower plasmin activity than rabbit plasma. Staphylococci that possess staphylokinase and staphylococcal Müller factor can activate the plasminogen-plasmin system in rabbit plasma and form plasmin, which in turn can cause fibrinolysis of the clot (false negatives). Some late fibrinolysis did occur in this study only in the citrated rabbit plasma. The clot-forming abilities of the two plasmas were basically identical when performing the tube coagulase test. No definite superiority of pig or rabbit plasma was observed.

Pig plasma, due to its availability and its comparable results with rabbit plasma, should be accepted in performing the tube coagulase test in microbiological laboratories. The thermostable nuclease test should also be run parallel with the coagulase test to aid in the identification of the weak coagulase producers and as a control of plasma lot variations.

LITERATURE CITED

1. Barry, A. L., R. V. F. Lachica, and F. W. Atchison. 1973. Identification of *Staphylococcus aureus* by simultaneous use of the tube coagulase and thermonuclease tests. Appl. Microbiol. 25:496–497.
2. Lachica, R. V. F., C. Genigeorgis, and P. D. Hoeprich. 1971. Meta chromatic agar-diffusion methods for detecting staphylococcal nuclease activity. Appl. Microbiol. 21:585–587.
3. Lachica, R. V. F., K. F. Weiss, and R. H. Deibel. 1969. Relationships among coagulase, enterotoxin, and heat-stable deoxyribonuclease production by *Staphylococcus aureus*. Appl. Microbiol. 18:126–127.
4. Orth, D. S., L. R. Chugg, and A. W. Anderson. 1971.

Comparison of animal sera for suitability in coagulase testing. Appl. Microbiol. 21:420–425.

5. **Parisi, J. T., J. N. Baldwin, and M. Sottile.** 1973. Pour plate method for the detection of coagulase production by *Staphylococcus aureus*. Appl. Microbiol. 25:558–561.
6. **Rayman, M. K., C. E. Park, J. Philpott, and E. C. D. Todd.** 1975. Reassessment of the coagulase and thermostable nuclease tests as a means of identifying *Staphylococcus aureus*. Appl. Microbiol. 29:451–454.
7. **Sperber, W. H., and S. R. Tatini.** 1975. Interpretation of the tube coagulase test for identification of *Staphylococcus aureus*. Appl. Microbiol. 29:502–505.
8. **Subcommittee on Taxonomy of Staphylococci and Micrococci.** 1965. Recommendations. Int. Bull. Bacteriol. Nomencl. Taxon. 15:109–110.
9. **Turner, F. J., and B. S. Schwartz.** 1958. The use of a lyophilized human plasma standardized for blood coagulation factors in the coagulase and fibrinolytic tests. J. Lab. Clin. Med. 52:888–894.