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Coagulase and Deoxyribonuclease Activities of Staphylococci Isolated From Clinical Sources[1]

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A total of 504 clinical isolates of the family *Micrococcaceae* were tested for coagulase, deoxyribonuclease, clumping factor, and phosphatase to determine whether there is a correlation between the results of these tests and the pathogenicity of staphylococci. In the tests for coagulase production, it was found that either human or rabbit plasma could be used with broth cultures, whereas rabbit but not human plasma was satisfactory when microorganisms removed from solid culture medium were used. Deoxyribonuclease production correlated better than the fermentation of mannitol with coagulase production. The use of methyl green, Toluidine Blue O, or acridine orange offered no advantage over the use of HCl for detecting the production of deoxyribonuclease. Neither the presence of clumping factor nor the production of phosphatase correlated well with coagulase production. Strains of staphylococci that did not produce coagulase and deoxyribonuclease were isolated as frequently as, and from a greater variety of clinical sources than, strains which produced these substances. It is concluded that the production of coagulase and deoxyribonuclease are properties of staphylococci which are not necessarily indicative of potential pathogenicity of the organisms for man.

An investigation of the tests for coagulase, deoxyribonuclease (DNase), clumping factor, and phosphatase was undertaken to determine whether any of these in vitro tests might be useful for predicting potential pathogenicity of staphylococci for man. It was considered necessary to determine whether either human or rabbit plasma could be used in the slide test for clumping factor and in the tube test for coagulase. Cadness-Graves et al. (6) stated that with human plasma coagulase-negative strains do not give positive slide tests for clumping factor. Using human plasma and growth from solid medium Needham et al. (22) found approximately 95% correlation between the slide test for clumping factor and the tube test for coagulase, and observed that a few strains reversed their reactions when subcultured and subsequently retested. Decrease or loss of clumping factor was also observed by Steward and Kelly (32) after storage of cultures and frozen cell suspensions but not when the cultures were lyophilized. Knowledge of this change in properties is important in the maintenance of cultures for quality control.

Manuals for clinical microbiology are not consistent or sufficiently specific in their recommendations for performing the slide test for clumping factor or for performing the coagulase test. In the American Society for Microbiology *Manual of Clinical Microbiology*, Ivler stated that the slide test for clumping factor is not recommended for routine diagnostic work and would not describe it in his chapter on staphylococci (14). However, in the same manual, Paik described the procedure for the slide test and recommended confirming all negative tests with a tube test (24). This gives the impression that a positive slide test for clumping factor indicates a positive tube test reaction for coagulase. In the American Public Health Association manual of diagnostic procedures, Blair recommended either human or rabbit plasma for the tube test for coagulase with growth from solid medium and in the slide test for clumping factor (3). Confirmation of negative slide tests with tube tests was also recommended and it was pointed out that some skill is required in performing and reading the slide test. The DNase test was reported by Jarvis and Wynne to be helpful in the case of strains

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that give doubtful reactions in the coagulase test (15).

Although many authors have referred to the production of coagulase and DNase as indicative of the pathogenicity of staphylococci for man, an examination of the strains from clinical material does not appear to have been made recently.

MATERIALS AND METHODS

Cultures. Strains of staphylococci were isolated from clinical specimens submitted to the laboratory. To include numerous coagulase-negative cultures in the study, some strains of gram-positive cocci were isolated from blood-agar plates that had been exposed to room air during the routine monitoring of air in the operating rooms. The cultures were maintained on heart infusion agar (HIA) slants, and usually not more than two or three transfers were made before performance of the various tests.

Plasmas. Human plasma was removed from those blood specimens which had been collected in Vacutainers (lavender-colored stoppers) containing EDTA (either the sodium or potassium salt of ethylenediaminetetraacetic acid) for hematological studies. Usually 1 to 2 ml of plasma could be removed from each tube. Although the blood was not collected aseptically, the plasma was handled aseptically. The plasma from a number of tubes was removed to yield a pool of at least 150 ml. This was centrifuged at 2,000 rev/min for 15 min to remove the blood cells, and then was dispensed in 10 to 12-ml amounts into tubes with screw-cap tops and stored at −10 C until used. Just prior to use, a sufficient quantity of the plasma was thawed and the contents of each tube were mixed thoroughly.

Rabbit plasma was Difco Coagulase Plasma EDTA obtained in the desiccated form. After being reconstituted according to the manufacturer's directions, it was either used promptly or stored at −10 C.

Media. DNase test agar (DTA), from either Difco or BBL, was prepared according to the manufacturer's directions and dispensed into sterile plastic petri dishes (90 by 15 mm).

DTA-MG medium was prepared by adding 1 ml of 0.5% methyl green solution to each 100 ml of DTA medium prior to autoclaving at 121 C for 15 min. After cooling to 50 C, the medium was dispensed into sterile plastic petri dishes. A stock solution of 0.5 g of methyl green (Allied Chemical NA 0420) was prepared in 100 ml of deionized water and repeatedly extracted with equal volumes of chloroform in a separatory funnel until the chloroform became colorless, as recommended by Smith et al. (31). The stock solution of methyl green was stored at 4 C.

DTA-TBO medium was prepared by adding 10 mg of Toluidine Blue 0 (Allied Chemical NA 0641) to each 100 ml of DTA medium prior to autoclaving at 121 C for 15 min (29). After cooling to 50 C, the medium was dispensed into sterile petri dishes.

DTA-AO medium was similar to that described by Omenn and Friedman (23), and was prepared by adding 1 ml of a stock solution of 0.4% acridine orange (basic orange 14; Matheson, Coleman and Bell, AX 305) to each 100 ml of DTA medium before autoclaving. After cooling to 50 C, the medium was dispensed into sterile petri dishes.

Phenolphthalein diphosphate agar (PDP medium) was prepared by adding 2 ml of a 0.5% stock solution of phenolphthalein diphosphate sodium salt and 3 ml of sterile defibrinated sheep blood to each 100 ml of HIA which had been melted and cooled to 50 C; the PDP medium was dispensed into sterile plastic petri dishes. The stock solution was prepared by dissolving 0.5 g of phenolphthalein diphosphate sodium salt in 100 ml of deionized water which had been sterilized by filtering through a 0.22-μm membrane filter (Millipore Corp.); the stock solution was stored at −10 C (2).

HIA and heart infusion broth (HIB) were obtained from Difco. Both media were stated by the manufacturer to have a final *p*H of 7.4.

The media for fermentation tests were prepared by adding 1% glucose or 1% mannitol to purple broth base (Difco). These media were dispensed in 6-ml amounts into 16 by 150 mm tubes containing 10 by 35 mm inverted tubes, which had been fitted with Morton closures (Scientific Products no. T1390-16) and sterilized by autoclaving at 121 C for 15 min.

Tests. Tube coagulase tests with broth cultures were performed by adding 0.5 ml of overnight HIB culture to 0.5 ml of human or rabbit plasma (diluted to 1 part rabbit plasma and 4 parts HIB) and incubating the tubes in a water bath at 37 C. The tubes were examined after 1 to 2, 4, and 6 hr. Any coagulation of the plasma was recorded as a positive reaction. Tubes in which no coagulation occurred were incubated overnight, and a final reading was made after 24 hr. If coagulation occurred, it took place usually within 1 to 2 or 4 hr and was in the form of a firm or nearly firm clot. Rarely did a positive reaction occur during overnight incubation. The reacting mixtures were contained in sterile 13 by 100 mm tubes with stainless-steel closures (Scientific Products no. T1390-13).

Tube coagulase tests with growth from solid medium were performed by suspending some growth from an overnight HIA slant in 0.5-ml amounts of human and undiluted rabbit plasma with a platinum loop. The tubes were similar to those used in the coagulase test with broth cultures. Readings were made after 1 and 3 hr and after overnight incubation in a water bath at 37 C. Any coagulation of the plasma was recorded as a positive reaction.

Slide tests for the clumping factor were performed by mixing some growth from an HIA slant with a drop of 0.85% sodium chloride solution on a microscope slide. If the suspension remained uniform, a drop of human or rabbit plasma was added and rapidly mixed by gently rotating the slide. Clumping of the organisms within 10 sec was recorded as a positive reaction.

For determining the ability of the organisms to ferment glucose and mannitol, one tube of glucose broth and one tube of mannitol broth were inoculated with the growth from a HIA slant, placed in a

GasPak (BBL) anaerobic jar, and incubated at 36 C overnight. The production of acid was evidenced by the change in color of the bromocresol purple indicator from purple to yellow.

The test for phosphatase was made by inoculating plates of PDP medium with up to eight cultures with the growth from HIA slants. Inoculations were made by making a streak about 2 cm long with an inoculating needle. After overnight incubation at 36 C, the opened plates were held over an open ammonia bottle. The growth containing phosphatase-producing organisms turned a bright-pink color, whereas no color change took place in the absence of phosphatase.

The test for DNase was made by inoculating plates of DTA with up to eight cultures with the growth from HIA slants by making streaks about 2 cm long with an inoculating needle. After overnight incubation at 36 C, the plates were flooded with 1 N HCl (16). The production of DNase by the growing cultures was evidenced by a clear zone remaining around the growth; in the absence of DNase, the medium turned opaque.

In many cases, parallel tests for the production of DNase were made with DTA-MG, DTA-TBO, and DTA-AO media. Plates of DTA-MG and DTA-TBO were inoculated and incubated similarly to the DTA plates. On DTA-MG, the production of DNase by the growing cultures produced a clear zone around the growth; the medium in the absence of DNase remained green. On DTA-TBO, the production of DNase was indicated by a rose-pink zone around the growth; the medium in the absence of DNase remained blue. Plates of DTA-AO were inoculated with up to 8 cultures with the growth from tubes of HIB by making short streaks with an inoculating loop. After overnight incubation at 36 C, the opened plates were examined in a darkened room with ultraviolet light by use of a Mineralite, model SL2537, 9 w, 110 v, short-wave ultraviolet filter (Ultra-Violet Products, Inc., South Pasadena, Calif.). A zone of nonfluorescence surrounded the growing cultures which produced DNase, whereas the medium fluoresced brightly in areas where DNase had not been produced.

RESULTS

In Table 1 are summarized the results obtained by testing 224 strains of staphylococci grown in broth for coagulase production by use of human and rabbit plasmas in the tube test. Also included are the results obtained by using the growth of the organisms from solid medium for detecting the presence of clumping factor in the slide test and the production of coagulase in the tube tests with the two plasmas, the production of DNase on DTA, and the ability to utilize glucose and mannitol anaerobically.

All 224 strains of staphylococci produced coagulase as determined by the tube test with broth cultures and at least one of the plasmas; 223 of the 224 strains coagulated rabbit plasma, and 219 of the 224 strains coagulated human plasma. When growth from solid medium was used in the tube test, 221 of the 224 strains of staphylococci coagulated rabbit plasma but only 36 of the 224 strains coagulated human plasma. When some of the growth from the same cultures on solid medium used in the tube tests was used for the slide tests with the same lots of plasma, only 152 of the 224 strains clumped in rabbit plasma and 184 strains clumped in human plasma. It is evident that rabbit plasma is better than human plasma for performing the tube coagulase test with growth from either solid or liquid media. If human plasma has to be used for the coagulase test, only broth cultures should be used. The presence of clumping factor as detected in the slide test was not a good indicator of coagulase production, as only 68% of the 224 cultures reacted with rabbit plasma and 82% reacted with human plasma.

Only 2 (0.89%) of the 224 coagulase-positive strains of staphylococci failed to give a positive test for DNase. These two strains also failed to ferment mannitol, failed to give a positive coagulase test with rabbit plasma in the tube test with growth from solid medium, and did not possess clumping factor. The broth cultures coagulated human and rabbit plasma in the tube test (line 15, Table 1). These strains were isolated from air.

The broth cultures of 69 strains of micrococci failed to coagulate human and rabbit plasmas in the tube tests, and it is this property which characterizes this group of cultures summarized in Table 2. The reason for selecting as the most reliable results those obtained in the coagulase test in which equal amounts of broth culture and plasma were contained in a tube, as proposed by Cowan in 1938 (7), was that it was shown by Williams and Harper (35) to be the most satisfactory technique. Only one strain fermented glucose and mannitol (line 12, Table 2); this strain failed to produce DNase and coagulase by any of the methods of testing, and it did not possess clumping factor. Only three strains coagulated rabbit plasma in the tube test with growth from solid medium, but the numbers of false-positive reactions were much greater with human plasma and in the slide tests for clumping factor. Five of the 69 cultures produced DNase.

Only 200 of the 224 coagulase-positive cultures listed in Table 1 and 19 of the 96 coagulase-negative cultures listed in Table 2 produced phosphatase. Additional tests for phos-

TABLE 1. *Clumping factor, coagulase, and DNase tests with staphylococci*[a]

Line	No. of cultures	Clumping factor slide test		Coagulase tests				Anaerobic fermentation		DNase
				Solid medium		Broth medium				
		H	R	H	R	H	R	G	M	
1	16	+	+	+	+	+	+	+	+	+
2	127	+	+	−	+	+	+	+	+	+
3	9	+	−	+	+	+	+	+	+	+
4	7	−	−	+	+	+	+	+	+	+
5	29	−	−	−	+	+	+	+	+	+
6	18	+	−	−	+	+	+	+	+	+
7	7	+	+	−	+	+	+	+	−	+
8	1	+	−	+	+	+	+	+	−	+
9	1	+	−	−	+	+	+	+	−	+
10	1	+	−	−	+	−	+	+	−	+
11	1	+	+	−	+	−	+	+	+	+
12	1	+	−	+	+	−	+	+	+	+
13	1	+	−	−	+	+	−	+	+	+
14	1	−	−	−	+	+	+	+	−	+
15	2	−	−	+	−	+	+	+	−	−
16	1	−	−	−	+	−	+	+	+	+
17	1	+	+	−	−	−	+	+	+	+
Total reacting strains	224	184	152	36	221	219	223	224	211	222

[a] H = human plasma; R = rabbit plasma; G = glucose; M = mannitol; + = clumping in the slide tests for clumping factor, coagulation in the tube tests for coagulation production, only acid produced in the fermentation tests, and production of DNase in the DNase test; − = no reaction.

TABLE 2. *Clumping factor, coagulase, and DNase tests with Micrococcaceae*[a]

Line	No. of cultures	Clumping factor slide test		Coagulase tests				Anaerobic fermentation		DNase
				Solid medium		Broth medium				
		H	R	H	R	H	R	G	M	
1	11	−	−	−	−	−	−	−	−	−
2	22	−	−	−	−	−	−	+	−	−
3	5	+	−	−	−	−	−	+	−	−
4	5	+	+	−	−	−	−	−	−	−
5	2	−	−	−	+	−	−	+	−	−
6	1	+	+	+	−	−	−	−	−	−
7	3	+	+	−	−	−	−	+	−	−
8	1	+	−	−	−	−	−	+	−	−
9	1	+	−	−	−	−	−	−	−	−
10	3	−	−	+	−	−	−	−	−	−
11	1	−	−	−	+	−	−	−	−	−
12	1	−	−	−	−	−	−	+	+	−
13	7	−	−	+	−	−	−	+	−	−
14	4	−	−	−	−	−	−	+	−	+
15	1	−	−	+	−	−	−	+	−	+
16	1	+	+	+	−	−	−	+	−	−
Total reacting strains	69	17	10	13	3	0	0	47	1	5

[a] See footnote *a* of Table 1.

phatase production were discontinued since the correlation between it and coagulase production was poor.

Since the results of the slide test for clumping factor with human plasma and growth from solid medium correlated poorly with positive reactions in the coagulase test with broth cultures and DNase production (Table 1), and also correlated poorly with negative reactions in these same tests (Table 2), additional cultures were not tested for clumping factor or for coagulase production with growth from solid medium and human plasma. Only tube tests were employed with rabbit plasma and growth from solid medium and human and rabbit plasmas with broth cultures for coagulase production in addition to tests for fermentation of glucose and mannitol and for DNase production with 211 cultures. The results are summarized in Table 3.

Again, there was good agreement in the results obtained in the coagulase tests with rabbit plasma and growth from either solid or liquid media (76 positive reactions) and with human plasma and broth cultures (77 positive reactions). Only 65 strains fermented glucose and mannitol, and with 20 strains (line 2, Table 3) the failure to ferment mannitol must be considered as serious negative correlations because the strains produced coagulase by the three methods of testing and produced DNase.

There were 23 among the 211 strains that produced DNase but failed to coagulate rabbit plasma. Strains with this combination of reactions are included in the strains cited in column 8 of Table 4, and their sources are discussed later.

Of 210 strains of staphylococci and micrococci tested for DNase production by two methods, 99 strains gave a positive reaction on DTA whereas only 76 were positive on the DTA-AO when examined with ultraviolet light. Similarly, when DTA and DTA-TBO were tested with 110 strains, 70 were positive for DNase on DTA but only 63 were positive on DTA-TBO. The DTA-AO and DTA-TBO appeared too insensitive for detecting DNase production, so comparative tests were discontinued.

In the comparison of DTA and DTA-MG with 258 strains, 165 were positive for DNase production and 81 were negative on both media. However, there were 12 strains that were negative on DTA but gave a positive or slightly positive reaction on DTA-MG. The latter medium gave too many slightly positive reactions that were difficult to interpret, so the use of DTA with the subsequent flooding of the medium with hydrochloric acid remained the method of choice for testing for DNase production.

DISCUSSION

Bergey's Manual (7th ed.) stated that the distinctive characters of *Staphylococcus aureus* are that the organisms are normally coagulase-positive (human or rabbit plasma) and ferment

TABLE 3. *Coagulase and DNase tests with staphylococci*[a]

Line	No. of cultures	Tube test			Anaerobic fermentation		DNase
		Broth medium		Solid medium			
		H	R	R	G	M	
1	55	+	+	+	+	+	+
2	20	+	+	+	+	−	+
3	1	+	−	−	+	+	+
4	1	+	−	−	+	−	+
5	1	−	−	+	+	+	+
6	1	−	+	−	+	−	−
7	20	−	−	−	+	−	+
8	8	−	−	−	+	+	−
9	99	−	−	−	+	−	−
10	5	−	−	−	−	−	−
Total reacting strains	211	77	76	76	206	65	98
Grand totals[b]	504	296	299	300	477	277	325

[a] See footnote *a* of Table 1.
[b] For Tables 1-3.

TABLE 4. *Summary of the reactions of 504 strains of Micrococcaceae in the tests for coagulase, DNase, and fermentation of glucose and mannitol*

Test	Columns and combinations of reactions[a]											
	1	2	3	4	5	6	7	8	9	10	11	12
Coagulase[b]	+	−	+	+	−	+	−	−	−	+	+	−
DNase[c]	+	−	+	−	−	−	−	+	+	−	+	+
Glucose[d]	+	−	+	+	+	−	+	+	+	+	−	−
Mannitol[d]	+	−	−	−	−	−	+	−	+	+	−	−
Total strains (504)	267	26	31	5	138	1	9	26	1	0	0	0

[a] Symbols: + = positive reaction; − = no reaction on the substrate.
[b] Coagulation of rabbit plasma in the tube test with growth from either solid or liquid medium.
[c] Production of DNase on DTA when tested with 1 N HCl.
[d] Production of acid during anaerobic incubation for 24 hr.

both glucose and mannitol under anaerobic conditions. Evans (10, 11) stressed the importance of anaerobic incubation of fermentation tests with mannitol and reported that each of 19 coagulase-positive (by rabbit plasma) strains of staphylococci fermented mannitol but 4% of 66 coagulase-negative strains also fermented mannitol. In a more extensive study, Mossel (21) reported that 2.1% of 389 coagulase-positive strains failed to ferment mannitol and 4.8% of 188 coagulase-negative strains fermented mannitol (anaerobically). Kimler (19, 20) found that about 1% of his strains of staphylococci were coagulase-positive and mannitol-negative and about 8% were coagulase-negative and mannitol-positive. He stated that based on 112 coagulase-positive and 49 coagulase-negative strains there was better agreement with mannitol fermentation and other reactions in the case of the coagulase-positive strains than in the case of coagulase-negative strains. Baird-Parker (1) observed a rather high frequency of coagulase-negative strains that utilized mannitol anaerobically, 2 among 40 strains. Person et al. (25) reported that 1.6% of 556 strains of *Micrococcaceae* were coagulase-positive and mannitol-negative, and 3.2% were coagulase-negative and mannitol-positive.

We found 35 (12%) of 304 coagulase-positive strains that failed to ferment mannitol and 10 (5%) of 200 coagulase-negative strains that fermented mannitol anaerobically. Thus, the incidence of coagulase-negative mannitol-positive strains is within the range reported by other investigators working with micrococci from human sources. Our incidence of 12% coagulase-positive mannitol-negative strains is higher than those reported by other workers with human strains. The difference may be due in part to the different methods of anaerobiosis used and the periods of observation. We read the fermentation tests after 24 hr of incubation, the same as for the DNase and the tube coagulase tests. If the mannitol tests had been incubated longer, as some investigators did, we may have obtained fewer negative reactions.

Brown et al. (5), working with staphylococci of bovine origin, reported 13 (25%) of 52 coagulase-positive strains that failed to ferment mannitol and only 1 of 191 coagulase-negative strains that fermented mannitol. Whether the organisms are from human or bovine sources, the production of coagulase and the anaerobic utilization of mannitol by staphylococci are not strictly parallel reactions.

There are 16 theoretical combinations of four reactions taken four at a time, but, since the combination of fermentation of mannitol and nonfermentation of glucose is not known to exist, there are only 12 possible combinations, as listed in Table 4. Approximately 88% of the 304 coagulase-positive strains produced coagulase and DNase and fermented glucose and mannitol; 13% of the 200 coagulase-negative strains gave negative reactions in each of the four tests (columns 1 and 2, Table 4). The other combinations of reactions shown by 211 (41.8%) of the 504 strains require some evaluations.

No strains were encountered with the combinations of reactions listed in columns 10, 11, and 12 (Table 4). The fermentation of glucose and negative reactions in the other three tests, as shown by the 138 strains cited in column 5, indicate that the single reaction, the fermentation of glucose, has little value in the identification of staphylococci. However, the inability to ferment glucose is helpful in ruling out staphylococci and potentially pathogenic micrococci, as will be seen when the 26 strains cited in column 2 are discussed. The 31 strains listed in column 3 can be considered as giving

atypical reactions with mannitol since the other three reactions were positive. The one strain listed for the reactions in column 6 may be considered as giving an atypical positive coagulation reaction since there were negative reactions in the other three tests; this strain was isolated from the air. The one strain listed for the combination of reactions in column 9 may be considered as giving an atypical negative reaction for coagulase since the reactions in the other three tests were positive; this strain was isolated from a skin ulcer.

The five cultures with the reactions listed in column 4 may represent atypical negative reactions for DNase and mannitol fermentation, as two of the cultures were isolated from abscesses, one from urine, and two from operating room air. The nine cultures with the reactions listed in column 7 may represent atypical negative reactions for the production of both coagulase and DNase if these strains are to be considered potentially pathogenic. The strains were isolated from the following sources: blood, boil, abscess, nose, and skin, one each; cervix, two; and urine, two.

There may be some justification for considering the majority of the 26 strains with the reactions listed in column 8 as potentially pathogenic for man but giving atypical negative reactions for coagulase production when tested with rabbit plasma and for mannitol fermentation. Zierdt and Golde (36) reported that 8% of their DNase-positive strains gave reactions of this type. A negative mannitol fermentation reaction is more likely to be associated with a negative coagulase reaction, as 95% of 200 coagulase-negative strains were mannitol-negative whereas only 88% of 304 coagulase-positive strains were mannitol-positive. Two of the cultures produced coagulase when tested with human plasma; one culture was isolated from air (Table 2, line 15) and the other from an eye (Table 3, line 4). The four strains listed on line 14 of Table 2 were isolated from operating room air; one strain consisted of large gram-positive cocci and in one strain tetrad arrangements predominated. It is possible for staphylococci to exhibit tetrad arrangements and for some strains of *Gaffkya* to be pathogenic. The sources of the 20 cultures listed on line 7 of Table 3 were as follows: abscess, 2; blood, 3; wound, 4; catheter tip, 2; nose, 1; sputum, 1; skin, 1; urethra, 1; urine, 2; peritoneal drainage, 1; penile discharge, 1; and frontal lobe, 1.

In view of the clinical sources of the 27 cultures cited in columns 8 and 9 of Table 4, and mentioned above, it may be advisable to give more serious consideration to the production of DNase, rather than the tests for coagulase production and the fermentation of mannitol, as an in vitro reaction indicative of potential pathogenicity of staphylococci for man. DNase production agreed slightly better with coagulase production than did mannitol fermentation in that of the 304 coagulase-positive cultures 98% produced DNase whereas 88% fermented mannitol. In addition 13.5% of the 200 coagulase-negative cultures produced DNase and only 5% fermented mannitol. Considering the many reports of infection in man by coagulase-negative strains of staphylococci (*S. epidermidis*, *S. albus*), a more critical in vitro test is needed for an indicator of potential pathogenicity, if such a reliable test is possible.

In performing the tube coagulase tests, the addition of 0.5 ml of overnight broth culture, as recommended by Jenkins and Metzger (18), or 0.1 or 0.2 ml of the culture, as recommended by Fisk (13), appeared to make no detectable difference in the outcome of the test. Our finding that, of 504 strains of micrococci, 299 were coagulase-positive with rabbit plasma and 296 were coagulase-positive with human plasma in the tube test with broth cultures is at great variance with the results of Jenkins and Metzger (18), who reported only 40% of the staphylococci coagulase-positive with rabbit plasma gave a positive reaction with human plasma. Our results are in agreement, however, with those of Jeljaszewicz (17), who found the two plasmas comparable and recommended that broth cultures of staphylococci be used for testing for free coagulase.

In contrast to broth cultures, the growth from solid medium gave widely discrepant results with human and rabbit plasmas in the tube test. Of the 223 cultures that were coagulase-positive in the tube test with broth cultures and rabbit plasma, 221 were coagulase-positive in the tube test with rabbit plasma and growth from solid medium but only 36 were positive with human plasma (Table 1). A possible explanation for the failure of many cultures to produce coagulase when the growth from solid medium is suspended in human plasma is that there may be antibodies or other substances in the human plasma that inhibit the production of coagulase. Streitfeld et al. (33) demonstrated that human gamma globulin inhibited coagulase production but did not inhibit growth. Ehrenkranz et al. (9) found that human sera may exert a bacteriostatic action on some strains of staphylococci, both coagulase-producers and non-producers. A given serum may inhibit the growth of some

coagulase-positive strains of staphylococci and not other strains (12). The possibility of the presence of antibiotics in blood freshly drawn from hospital patients and used in the coagulase tests, which could inhibit the metabolism of staphylococci and the production of coagulase, cannot be overlooked. The use of broth cultures and diluted plasma could obviate these difficulties.

The DNase produced by *S. aureus* was reported by Cunningham et al. (8) as being distinctive in that, among other things, it was heat-stable. In testing a group of staphylococci, the majority of which produced enterotoxin, Victor et al. (34) found 95% of 275 coagulase-positive strains to produce the heat-stable DNase. More important was the finding among this selected group of staphylocci that 24.4% of 41 coagulase-negative strains also produced the heat-stable DNase. Coagulase-negative enterotoxin-positive staphylococci have produced outbreaks of food-borne gastroenteritis, so there is not a definite correlation of coagulase and enterotoxin production by staphylococci (4).

The negative coagulase tests for the 36 strains listed in columns 7, 8, and 9 of Table 4 do not appear to be criteria for nonpathogenicity as discussed above. However, 27 of the 36 strains produced DNase. Comprising the five cultures with the reactions in column 4 of Table 4 were two cultures from abscesses, one from urine, and two from air. Thus, the failure to produce DNase does not appear to be an absolute criterion for nonpathogenicity.

Considering the 26 strains cited in column 2 of Table 4, it appears that the failure to utilize glucose anaerobically is an important reaction in ruling out staphylococci and potentially pathogenic *Micrococcaceae*, as only four of the strains were isolated from urine, one from sputum, and one from a furuncle. One strain was *Micrococcus roseus* and the remainder of the cultures were from plates that had been exposed to room air.

One would expect the sources of the strains cited in column 1, which gave a positive reaction in all four tests, to be from the most severe infections, but that was not the case. The greatest proportion of the strains came from sputum and nasopharyngeal and throat swabs, some from wounds and ulcers, and a few from abscesses, carbuncles, cervix, blood, urine, skin, and air. The greatest surprise was the wide distribution of the strains which produced neither coagulase or DNase (column 5, Table 4). Blood and wound cultures contributed the greater numbers of these strains with the following sources each contributing a few strains: eye, ear, urine, cervix, cerebrospinal fluid, abscesses, sinus, skin, pus, lymph nodes, subclavian catheter tip, trachea, ulcer, pericardial fluid, fistula, masses in the neck and breast, penile abrasion, and air.

The sources of the cultures cited in column 3 were as varied as, and comparable to, the sources of the strains cited in column 5, even though one group of cultures produced both coagulase and DNase and the other group produced neither one. The sources of the cultures in column 5 were as follows: wounds, nine; pharynx, four; blood, three; abscesses, two; throat, two; and one strain each from cerebrospinal fluid, tracheal aspirate, spleen, knee, eye, rash, skin, lochia, and synthetic intravenous solution.

It becomes readily apparent that the production of coagulase, as detected with broth cultures and human or rabbit plasma or growth from solid medium and rabbit plasma, and the production of DNase are properties that may be useful for identifying the species *S. aureus*, but these properties should not be taken as in vitro reactions indicative of pathogenicity, nor is their absence indicative of nonpathogenicity. The division of staphylococci into *aureus* and *epidermidis* species was questioned by Smith and Farkas-Himsley (30). The fact remains there is no reliable in vitro test for determining potential pathogenicity of staphylococci for man. In clinical microbiology, the signs and symptoms of the patient provide the pathogenicity test for the culture isolated in the laboratory. Numerous investigators over a period of many years have reported many instances where coagulase-negative strains, the so-called *S. albus* or *S. epidermidis*, have produced serious infections in man. These infections have included subacute bacterial endocarditis (22, 26) and bacteremia associated with ventriculovenous-cerebrospinal fluid shunt (26, 27) and ventriculoatriostomy (28).

The current trend in the management of patients, such as the use of circulatory prostheses, venous catheters, and immunosuppresent drugs, are factors which have a bearing on whether strains of staphylococci are pathogenic, and not everything depends upon a particular biochemical reaction of the organisms.

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