

Deoxyribonuclease-Positive *Staphylococcus epidermidis* Strains

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Received for publication 13 March 1970

Use of the agar plate test for the enzyme deoxyribonuclease 3'-nucleotidohydrolase (deoxyribonuclease) can result in frequent misdiagnosis of *Staphylococcus epidermidis* as *S. aureus*.

The distinction between *Staphylococcus aureus* and *S. epidermidis* is one of the most important differential determinations to be made in the clinical laboratory. Of the numerous diagnostic reactions available to identify potentially pathogenic staphylococci, the test for coagulase has proved the most useful. In 1957, Weckman and Catlin (12), using a tube viscometric method, demonstrated that *S. aureus* produced large quantities of the enzyme ribonuclease (deoxyribonuclease) 3'-nucleotidohydrolase (deoxyribonuclease). *S. epidermidis* demonstrated significantly lower deoxyribonuclease activity. It was suggested that deoxyribonuclease production might be useful as a supplementary determinative characteristic. A plate test for the detection of bacterial nuclease was later developed by Jeffries and co-workers (9). Burns (1) reported the deoxyribonuclease plate test to correlate well with coagulase production, pigmentation, and mannitol fermentation by staphylococci but found 11 of 49 strains of *S. epidermidis* to be deoxyribonuclease positive. Subsequently, DiSalvo (4) reported 100% correlation between tube coagulase and deoxyribonuclease plate tests in strains of coagulase-positive and coagulase-negative staphylococci.

During bacteriophage typing of *S. aureus* which had been differentiated from *S. epidermidis* by a positive deoxyribonuclease-agar test, phage nontypable strains were encountered which exhibited an unusual bacterial lawn morphology and a negative coagulase test. The purpose of the present communication is to report on the properties of these strains and on the differential utility of the deoxyribonuclease-agar plate test.

MATERIALS AND METHODS

Deoxyribonuclease-positive *Staphylococcus* isolates were obtained from cultures of blood, skin, sputum, and stool. Thirty strains with the atypical, rough lawn pattern (Fig. 1) were selected for study. Strains ex-

hibiting this rough growth were white or light yellow and all were phage nontypable. O-F (Difco) and Phenol Red Broth Base (Difco) media were used to test mannitol and dextrose fermentation. Urease production was tested on Christensen's urea agar slants. The organisms were grown on Tellurite Glycine Agar (Difco) and Egg Yolk Agar (Difco). Hemolysis was assessed on sheep blood-agar. Slide and tube coagulase tests were performed with ethylenediaminetetraacetic acid plasma (Difco), and gelatinase was tested by the Kohn method (10). Enterotoxin production (types A, B, C, and D) was assayed by microimmunodiffusion (3).

The deoxyribonuclease plate test was performed on agar composed of 0.2% deoxyribonucleic acid (DNA), 2% Tryptose (Difco), 0.5% NaCl, and 1.5% agar (Difco formula). Overnight cultures were spot-inoculated on the agar surface. After incubation at 37 C for 16 to 24 hr, the plate was flooded with 1 N HCl. Precipitation of unhydrolyzed DNA produced an opaque milky appearance. Where DNA was hydrolyzed by bacterial nuclease, a transparent zone remained (Fig. 2). Any clear zone around the growth was considered a positive test.

Three special DNA agars were prepared with 0.2% DNA of different purity grades. Standard and special DNA plates were inoculated with 100 freshly isolated strains of coagulase-positive *S. aureus* and the 30 atypical strains of *S. epidermidis*. These strains were tested after 18 hr of incubation at 30 C.

RESULTS

Sixteen per cent of the strains submitted as *S. aureus* on the basis of a positive deoxyribonuclease plate test were found to be nontypable at the 100 routine test dose concentration of the typing set of 22 phages (3B/3C/6/7/29/42B/42D/42E/44A/47/52/52A/53/54/55/71/77/80/81/83A/86/187). Half of the nontypable strains (8%) exhibited the atypical lawn morphology and were coagulase-negative. Biochemical testing of these strains (Table 1) indicated that the organism was in fact *S. epidermidis*. The remainder of the nontypable strains were typical coagulase-positive *S. aureus*. Repeat deoxyribo-

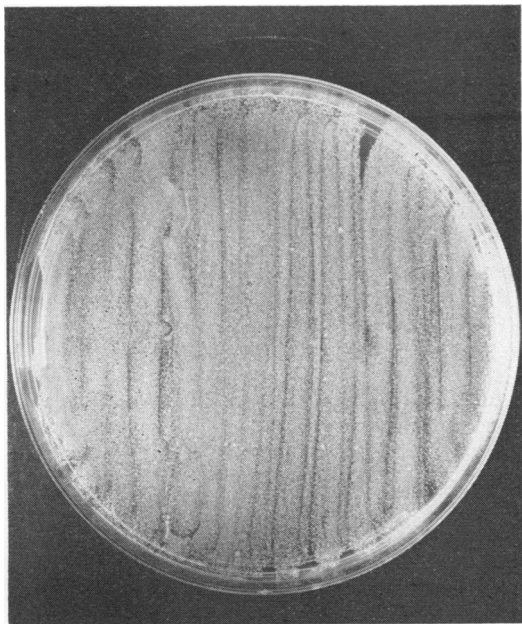


FIG. 1. "Rough"-lawn type of *S. epidermidis*. Appearance of roughness is partially due to failure of discrete colonies to coalesce as confluent growth and partially to dull, semimatt colonies.

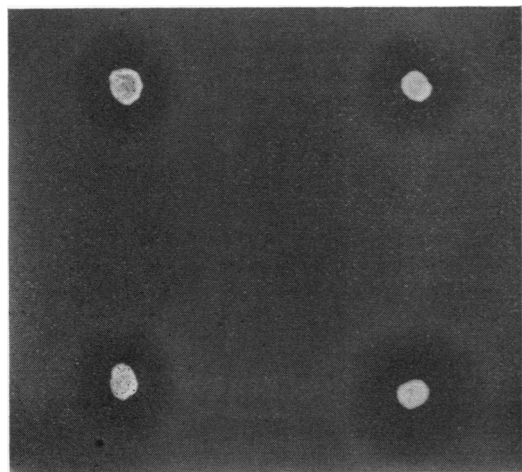


FIG. 2. Growth of four atypical *S. epidermidis* strains (24 hours) on deoxyribonuclease-agar, surrounded by clear zones indicative of DNA hydrolysis.

nuclease plate tests performed on the 30 atypical strains yielded only 20 positive reactions. This discrepancy was later found to represent natural variation in these strains, probably related to growth on unenriched media. When the organisms were grown on sheep blood-agar and then

tested on the standard deoxyribonuclease plate, 27 positive reactions were observed. Also, addition of niacin and thiamine (5 µg/ml) to the plates caused a marked increase in the number of positive reactions.

Testing with the special deoxyribonuclease-agar plates yielded more positive results when higher purity DNA was used (Table 2). Crude herring sperm DNA (Mann Research Laboratories) provided an insensitive test for nuclease production, whereas purified DNA (Sigma Chemical Co.) gave 100% positive reactions and large zones with the atypical strains.

Three strains of *S. epidermidis*, ATCC 12228, ATCC 17917, and ATCC 14990, the proposed neotype strain (6), produced no zones on standard or high-purity deoxyribonuclease-agar (Fig. 3), even after 72 hr of incubation. One hundred strains of known *S. aureus* gave positive reactions on all deoxyribonuclease plates.

None of the 30 atypical strains was hemolytic on sheep blood-agar and none produced enterotoxin (Table 1). All of the *S. aureus* strains were hemolytic. In a previous study, about 50% of randomly selected strains from this hospital were positive for one or more of the enterotoxins.

DISCUSSION

The production of nuclease by staphylococci can be qualitatively detected by the deoxyribonuclease-agar plate test. This test has been standardized for clinical purposes to distinguish *S. aureus* from *S. epidermidis*. Both organisms are known to elaborate deoxyribonuclease, but *S. aureus* produces the enzyme in much greater amounts. The test therefore depends on a quantitative discrimination.

Various workers have compared the deoxyribo-

TABLE 1. Biochemical reactions of 30 atypical strains found to be *S. epidermidis* versus *S. aureus* strains

| Determination | 30 Atypical strains | Control <i>S. aureus</i> strains |
|-------------------------|---------------------|----------------------------------|
| Dextrose fermentation | + | + |
| Mannitol | - | + |
| Catalase | + | + |
| Urease | - | + |
| Coagulase | - | + |
| Egg yolk | - | + |
| Tellurite | - | + |
| Gelatinase | - | + |
| Hemolysis (sheep blood) | - | + |
| Enterotoxin | - | - ^a |

^a Variable.

TABLE 2. Deoxyribonuclease reactions of rough-lawn *S. epidermidis* and of *S. aureus* on DNase agars of different formulation^a

| Organism | Positive reactions | | | | | |
|---|--------------------|------------------|--------------------------------------|------------------------|-----------------------|----------------------|
| | Inoculum grown on | Difco DNase Agar | Difco DNase Agar + niacin + thiamine | Difco base + Sigma DNA | Difco base + Mann DNA | Difco base + NBC DNA |
| Rough-lawn <i>S. epidermidis</i> (30 strains) | TSA ^b | 20 | 30 | 30 | 14 | 16 |
| | Sheep blood-agar | 27 | 30 | 30 | 23 | 24 |
| <i>S. aureus</i> (100 strains) | TSA | 100 | 100 | 100 | 100 | 100 |
| | Sheep blood-agar | 100 | 100 | 100 | 100 | 100 |

^a Sigma Chemical Co. DNA type I: highly polymerized, derived from calf thymus; highest grade. Nutritional Biochemicals Corp. (NBC) DNA: not highly polymerized; intermediate grade. Mann Research Laboratories DNA: derived from herring sperm; crude commercial grade.

^b Trypticase Soy Agar.

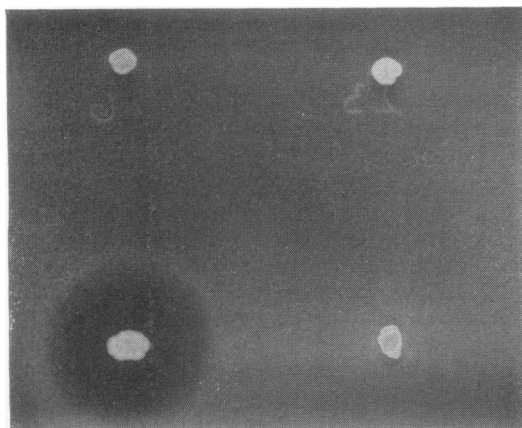


FIG. 3. Negative deoxyribonuclease reactions of ATCC 12228 (upper left), ATCC 17917 (upper right), ATCC 14990 (lower right). Lower left, deoxyribonuclease-positive *S. aureus* control.

nuclease plate test with other methods of distinguishing potentially pathogenic staphylococci, and often divergent results have been reported. DiSalvo (4) found 100% correlation between the tube coagulase and deoxyribonuclease plate tests in 304 strains of staphylococci. Other investigators (1, 7), however, have reported between 2.2 and 22.4% of strains of *S. epidermidis* to be deoxyribonuclease-positive. Franklin et al. (5) reported 850 of 3,101 strains of coagulase-negative cultures to give a positive deoxyribonuclease reaction and 211 of 6,260 coagulase-positive cultures to give negative deoxyribonuclease tests. Pomorski (M. S. Thesis, Wayne State Univ., Detroit, Mich., 1962), using a modification of the

deoxyribonuclease plate test, found 36% of *S. epidermidis* strains to be deoxyribonuclease-positive.

In testing strains of *S. aureus* which had been distinguished from *S. epidermidis* solely by a positive standard deoxyribonuclease plate test, we found 8% to be coagulase-negative and phage nontypable. Further biochemical testing showed that the strains had been misdiagnosed and were in fact *S. epidermidis*. Reactions on deoxyribonuclease-agar plates were found to be influenced by the medium from which the organism was isolated, the presence of niacin and thiamine, and, most importantly, the purity grade of DNA used.

The 30 strains of *S. epidermidis* reported here had group characteristics other than deoxyribonuclease positivity that permitted their identification. These atypical strains demonstrated flocculent growth in broth and a rough growth on agar that consisted of closely packed discrete matt colonies. Subsequently, we have found proven *S. epidermidis* strains not exhibiting this atypical growth which were also deoxyribonuclease-positive. However, the majority of strains misdiagnosed as *S. aureus* were of the atypical type.

The agar-deoxyribonuclease test, because of the lengthy incubation period of 18 to 24 hr, elicits positive reactions from relatively small amounts of nuclease. At the same time there is a possibility of DNA breakdown due to enzymes other than deoxyribonuclease or metabolic products such as organic acids. The tube viscometric method of Weckman and Catlin (12) and the tube spectrophotometric method of Cuatrecasas et al. (3) obviate some sources of error. It is doubtful whether the agar test for nuclease is of high validity even for qualitative evaluation. Plates

prepared with a lower purity grade DNA provide a less sensitive test for deoxyribonuclease but discriminate better between *S. aureus* and *S. epidermidis*, because small amounts of deoxyribonuclease produced by *S. epidermidis* will not be detected.

No attempt was made to correlate deoxyribonuclease activity of the special group of *S. epidermidis* strains with pathogenic potential. All of the atypical *S. epidermidis* strains in this study were isolated from clinical material and some of them were causing infection. There is increasing evidence that deoxyribonuclease production in staphylococci correlates with pathogenic potential (7, 8). The use of the deoxyribonuclease-agar plate test as a basis for distinguishing *S. aureus* from *S. epidermidis*, however, will result in an unacceptably high incidence of misdiagnosis.

ACKNOWLEDGMENTS

Ezra Casman kindly examined the atypical strains for enterotoxin production. Sylvia Gersch provided excellent technical assistance.

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