APPLIED MICROBIOLOGY, July 1969, p. 95-97
Vol. 18, No. 1

Printed in U.S.A.

# Characterization of *Micrococcaceae* Isolated from Clinical Sources

DONALD A. PERSON, PAULINE K. W. YU, AND JOHN A. WASHINGTON, II
*Section of Microbiology, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901*

Received for publication 18 April 1969

The characterization of 556 *Micrococcaceae* isolates from various clinical sources has been presented. The incidence of coagulase-negative mannitol-positive staphylococci was 3.2% and that of coagulase-positive mannitol-negative staphylococci was 1.6%. There appears to be insufficient justification for the routine reporting of staphylococcal variants from a general bacteriology laboratory, and biotyping should be reserved for special circumstances.

In the last several years, a number of investigators have studied members of the family *Micrococcaceae* with respect to their physiological and biochemical activity (1, 2, 5, 8, 9). Most of these investigations have dealt with organisms from nonhuman sources. The problem of pathogenicity of coagulase-negative staphylococci has not been well defined, although it is generally agreed that these organisms are associated with disease states in certain circumstances (3, 11). Attempts at biotyping these organisms may prove to be most helpful; however, with the multiplicity of tests required, the usefulness of these methods in a clinical diagnostic laboratory is limited (3). Recently, Branson (4) reported the results of limited biotyping of 220 *Micrococcaceae* isolates collected over a 15-week period in a private general hospital and found that almost 18% of *Staphylococcus aureus* strains isolated were coagulase-negative.

In an attempt to define the frequency in this laboratory of coagulase-negative mannitol-positive staphylococci, 556 primary clinical isolates were selected for study.

## MATERIALS AND METHODS

Strains to be studied were randomly chosen from the Diagnostic Bacteriology Laboratory during the months of August through November 1968 and in January 1969. Colonies selected for study were picked from Blood-Agar plates (BBL) which had been incubated at 37 C for 18 to 24 hr. Colonial morphology and hemolysin production were noted, and a Gram stain was performed on all isolates. Each strain was tested for catalase activity and nitrate reduction. Coagulase activity was determined by slide and tube tests utilizing rabbit plasma (Difco). Fermentation reactions were determined by inoculation of a 1% mannitol semisolid agar-stab tube and a 1% glucose semisolid agar-stab tube that were overlaid with 3 to 5 cm of sterile mineral oil and incubated at 37 C for 24 or 48 hr. Strains (378) were tested for deoxyribonuclease activity (Deoxyribonuclease Test Medium, BBL).

## RESULTS

The source of the specimen, total number of isolates, and the results of the various tests performed are listed in Table 1. The agreement of the slide- and tube-coagulase tests was 92%. All isolates were catalase positive, and all strains reduced nitrates with the exception of *Gaffkya tetragena*. Coagulase-positive mannitol-negative staphylococci represented 1.6% of the total isolates (or 3.2% of strains defined as *S. epidermidis* on the basis of mannitol fermentation) and coagulase-negative mannitol-positive staphylococci represented 3.2% (or 8% of strains defined as *S. aureus* on the basis of mannitol fermentation). Of the 378 isolates tested for deoxyribonuclease activity, the agreement of the deoxyribonuclease test with the coagulase test was 97.8% for coagulase-positive strains and 94.1% for coagulase-negative strains.

## DISCUSSION

Mossel (9) found that 2.1% of coagulase-positive staphylococci were mannitol-negative, and, in Kimler's experience, this figure was 1 to 2% (6, 7). The incidence in this report is 3.2%. Kimler's observation that approximately 8% of coagulase-negative strains ferment mannitol (6) is in complete agreement with the results obtained in our study. This is in contrast to the 18% incidence found by Branson (4). Papaevangelou and Papavassiliou studied 328 strains of staphylococci and found that 98.4% of 262 coagulase-positive strains were deoxyribonuclease positive and that 96.9% of 66 coagulase-negative strains were deoxyribonuclease negative (10). These re-

TABLE 1. *Characterization of Micrococcaceae from clinical sources*

| Source of specimen | Total no. of isolates | *Staphylococcus aureus* | *S. epidermidis* | Coagulase-negative and mannitol-positive staphylococci | Coagulase-positive and mannitol-negative staphylococci | *Gaffkya tetragena* | *Micrococcus* | *Sarcina* |
|---|---|---|---|---|---|---|---|---|
| Ear, nose, throat, and mouth | 115 | 69 | 39 (2)[a] | 1 | 5 | 1 | | |
| Skin | 80 | 39 (1) | 38 (2) | | 3 | | | |
| Blood | 69 | 36 | 32 (3) | | | | 1 | |
| Urine | 68 | 3 (1) | 57 (3) | 5 (3) | | 3 | | |
| Wound and ulcer | 51 | 25 (1) | 23 | 3 | | | | |
| Sputum, bronchial washings, and tracheal aspirations | 34 | 15 | 16 (1) | 3 | | | | |
| Abscess | 22 | 12 | 6 | 4 | | | | |
| Eye | 22 | 7 | 15 (1) | | | | | |
| Urogenital (kidney, bladder, cervix, prostate, and lochia) | 18 | 1 | 15 (1) | 2 | | | | |
| Pleural and peritoneal fluid | 14 | 6 | 8 | | | | | |
| Synovial fluid | 13 | 8 | 5 | | | | | |
| Spinal fluid | 12 | | 11 | | 1 | | | |
| Heart valve | 11 | | 11 | | | | | |
| Fistulae (sinus tract, drain catheter tip, and cut-down site) | 10 | 2 | 8 | | | | | |
| Bone | 9 | 2 | 6 | | | 1 | | |
| Bile | 4 | | 4 | | | | | |
| Tissue (node, liver, and parotid gland) | 3 | 1 | 2 | | | | | |
| Pleural fluid of rabbit | 1 | | | | | | | 1 |
| Total | 556 | 226 | 296 | 18 | 9 | 5 | 1 | 1 |
| Per cent | 100.0 | 40.7 | 53.2 | 3.2 | 1.6 | 0.9 | 0.2 | 0.2 |

[a] Numbers in parentheses represent number of isolations giving a "false-negative" or "false-positive" deoxyribonuclease test (that is, *S. aureus* or *S. epidermidis* on the basis of mannitol fermentation).

sults agree rather closely with our figures of 97.8 and 94.1%, respectively.

Our data (8%) are at considerable variance with those of Branson (4) in which the incidence of coagulase-negative *S. aureus* is 18%. To explain this discrepancy is difficult. However, it is possible but unlikely that the variance is due to differences in sampling and differences in the source or sensitivity of the commercial rabbit plasma. Furthermore, there is a chance that in Branson's study an absence of true anaerobiosis in the test for mannitol fermentation may have enabled an oxidative breakdown to occur, resulting in a falsely high number of positive reactions. Whether Branson's mannitol fermentation reaction "at the bottom of the tube" (4) enhances this last possibility is not known. It is interesting to observe in the present study, as well as in Branson's (4), that a large proportion of the coagulase-negative mannitol-positive strains of staphylococci were isolated from the urine or urogenital tract. The explanation for this phenomenon bears further investigation. Excluding all urine isolates from both studies, however, does not eliminate the differences between the two studies; it only serves to reduce our own incidence of coagulase-negative mannitol-positive strains of *S. aureus* to 5% and Branson's (4) to 11%.

No attempt was made to ascribe clinical significance to each isolate in this study but rather to study isolates from a wide variety of clinical sources. Particular attention was directed to those isolates from sources in which it might be clinically important to determine if the organism represented a coagulase-negative mannitol-positive type of staphylococcus; hence there was a large

number of isolates from blood, normally uncontaminated body fluid, tissue, cerebrospinal fluid, abscesses, and wounds.

On the basis of this investigation, we cannot recommend the general adoption of multiple testing of staphylococci in the general medical microbiological laboratory except in unusual circumstances or for research purposes. At the present time there appears to be insufficient justification for routine reporting of staphylococcal variants. The decision to request sensitivity testing and to initiate treatment remains a clinical one.

ACKNOWLEDGMENT

Robert Birk provided valuable technical assistance.

LITERATURE CITED

1. Baird-Parker, A. C. 1963. A classification of micrococci and staphylococci based on physiological and biochemical tests. J. Gen. Microbiol. 30:409–427.
2. Baird-Parker, A. C. 1965. Staphylococci and their classification. Ann. N.Y. Acad. Sci. 128:4–25.
3. Bentley, D. W., R. Haque, R. A. Murphy, and M. H. Lepper. 1968. Biotyping, an epidemiological tool for coagulase-negative staphylococci. Antimicrobial Agents and Chemotherapy—1967, p. 54–59.
4. Branson, D. 1968. Identification of *Micrococcaceae* in clinical bacteriology. Appl. Microbiol. 16:906–911.
5. Brown, R. W., O. Sandvik, R. K. Scherer, and D. L. Rose. 1967. Differentiation of strains of *Staphylococcus epidermidis* isolated from bovine udders. J. Gen. Microbiol. 47:273–287.
6. Kimler, A. 1962. Some clinical laboratory briefs on staphylococci. J. Bacteriol. 83:207–208.
7. Kimler, A. 1962. Evaluation of mediums for identification of *Staphylococcus aureus*. Amer. J. Clin. Pathol. 37:593–596.
8. Kocur, M., and N. Mortensen. 1967. Comparison of methods for estimation of anaerobic production of acid from glucose and mannitol in staphylococci and micrococci. Acta Pathol. Microbiol. Scand. 71:141–146.
9. Mossel, D. A. A. 1962. Attempt in classification of catalase-positive staphylococci and micrococci. J. Bacteriol. 84: 1140–1147.
10. Papaevangelou, G., and J. Papavassiliou. 1967. Comparison of desoxyribonuclease activity to some other criteria of *Staphylococcus* pathogenicity. Pathol. Microbiol. 30:59–63.
11. Quinn, E. L., F. Cox, and M. Fisher. 1965. The problem of associating coagulase-negative staphylococci with disease. Ann. N.Y. Acad. Sci. 128:428–442.