Microbiological Study of Streptococcal Bacteremia

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During a 2.5-year study, streptococci were isolated from 280 patients (19% of those with bacteremia). Of this number, 54 had group D streptococci (45 were enterococci), 218 had α- or γ-hemolytic nongroup D streptococci, and 49 had β-hemolytic streptococci.

With the increased frequency in recent years of gram-negative bacillaeemia, there has been relatively less attention devoted to streptococcal bacteremia. Recent data obtained at the Mayo Clinic have shown that streptococci were isolated from 19% of patients with bacteremia. The advent of more definitive means of speciating some viridans streptococci (4) and group D streptococci (7) of human origin prompted us to review the distribution of groups and species isolated from blood cultures over a 2.5-year period.

MATERIALS AND METHODS

Blood culture procedures at the Mayo Clinic and affiliated hospitals have been described (17, 18). Since the fall of 1971, the media have contained 0.03% sodium polyanetholsulphonate (SPS). Otherwise, the basic system since 1968 has been to use two unvented, commercially available bottles, each containing 100 ml of liquid medium under vacuum and carbon dioxide. Each bottle is inoculated with 10 ml of aseptically collected blood. The bottles are examined later on the day of inoculation and daily thereafter for 14 days. A routine "blind" subculture to chocolate blood agar is performed within the first 24 h after inoculation. Cultures suspected of being positive on gross examination are immediately sampled for staining and also subcultured both aerobically and anaerobically.

Streptococci that are not strictly anaerobic are initially classified according to the hemolytic reaction on 5% sheep-blood agar, solubility in 10% sodium deoxycholate, and bile-esculin reaction. If an isolate is β-hemolytic, Lancefield grouping is performed with commercial antisera (Burroughs Wellcome) by the technique described by Rantz and Randall (13). In our experience, results obtained by this technique have agreed closely with those obtained at the Center for Disease Control (courtesy of R. R. Facklam) by the Lancefield acid extraction method (12). Isolates yielding a positive bile-esculin reaction are speciated by the techniques and classification described by Facklam (7). Viridans streptococci isolated from patients with endocarditis have been referred to R. R. Facklam at the Center for Disease Control for study.

Streptococci that produce dry, adherent colonies yield variably positive bile-esculin reactions, do not react in group D antiserum, ferment mannitol, and produce raised, white, crystalline colonies on 5% sucrose agar are presumptively called Streptococcus mutans (4).

RESULTS

The numbers of patients positive are shown in Table 1. Those viridans streptococci isolated from patients other than those with endocarditis were not further characterized. Of the 321 patients, 54 (17%) had group D streptococci, of which 45 represented enterococci (divisions I and II). Five non-enterococcal group D streptococci were identified as S. bovis. Four group D streptococci could not be speciated because their biochemical reactions would not fit any species description; two of these were salt tolerant and two were not. All but one of the group D streptococci yielded a positive bile-esculin reaction, defined as blackening of one-half or more of the medium within 72 h (8). Preliminary differentiation between enterococcal and non-enterococcal group D streptococci was accurately made on the basis of growth in 6.5% NaCl broth. Many of these patients had endocarditis.

Of the 218 (68%) patients with α- and γ-hemolytic nongroup D streptococci, 41 (18%) had pneumococci. Nearly all of the patients with S. mitis (20), streptococcus MG (1), S. sanguis (15), or S. mutans (9) had endocarditis. Of interest is a patient with postoperative septicemia, noted by Wilkinson et al. (19), from whose blood a nonhemolytic group B streptococcus was isolated in pure culture.

Also shown in the table are 49 patients with β-hemolytic streptococcal isolates. Group A streptococci were the most frequently isolated...
The table below shows the numbers of patients with streptococcal bacteremia, by species, November 1970 through April 1973. Streptococci, a heterogeneous group of organisms, are frequently named in published articles as a distinct species, S. viridans, which cannot be found in Bergey's Manual (3). In view of the number of tests required for speciation of these organisms, it is unlikely that most clinical laboratories will be able to do much more than provide a general classification.

In a bacteriological and clinical study of streptococcal bacteremia reported by Duma et al. (6) in 1969, hemolytic reactions were determined on brucella agar with 5% horse blood, and Lancefield grouping of all isolates was attempted. Because of uncertainties regarding the level of agreement between hemolytic reactions obtained with agar containing sheep or horse blood and the degree of reliability of Lancefield grouping of α- and γ-hemolytic streptococci, strict comparison of the bacteriological aspects of the two studies is difficult. In general, the predominance, in our study, of isolates in Lancefield groups A and D and in the nongroupable category corresponds with that observed by Duma et al. Because speciation of group D streptococci was not performed in their study, it is not possible to determine the relative frequencies of enterococcal and non-enterococcal bacteremias.

In a study of species differences in susceptibility of group D streptococci to various antibiotics, Toala et al. (16) isolated no non-enterococcal species from blood cultures. They recovered S. faecalis var. liquefaciens and S. faecalis var. zymogenes in division I and S. faecium in division II.

Presumptive identification of group D streptococci in blood cultures may be rapidly accomplished by subculture to bile-esculin agar (8). Although a selective enterococcus medium (PSE, Pfizer) may be useful for isolating group D streptococci, it has not been found to be reliable for presumptive identification of group D streptococci (Facklam, Appl. Microbiol., in press). Differentiation between enterococcal (divisions I and II) and non-enterococcal (division III) group D streptococci may be helpful clinically, because the latter are considerably more susceptible to antibiotics than the former (20). It may be accomplished reliably and easily by determining whether or not growth occurs in 6.5% NaCl, providing that the test has been properly standardized (R. R. Facklam, Bacteriol. Proc., p. 105, 1971). According to Deibel (5) and Facklam (7), differentiation of the two varieties of S. faecalis (liquefaciens and zymogenes) is sufficiently unreliable to warrant its discontinuation.

The importance of various serological groups

### Table 1. Numbers of patients with streptococcal bacteremia, by species, November 1970 through April 1973

| Organism          | No.  |
|-------------------|------|
| α- and γ-hemolytic|      |
| Group D           |      |
| Division I        |      |
| S. faecalis       | 42   |
| Division II       |      |
| S. faecium        | 2    |
| S. durans         | 1    |
| Division III      |      |
| S. bovis          | 5    |
| Unable to speciate| 4    |
| **Subtotal**      | 54 (17%) |
| Other than group D|      |
| S. pneumoniae     | 41   |
| S. mitis          | 20   |
| S. sanguis        | 15   |
| Strep. MG         | 1    |
| S. salivarius     | 9    |
| S. mutans         | 9    |
| Group B           | 1    |
| Group C           | 1    |
| Group F           | 1    |
| **"Viridans group"** | 120  |
| **Subtotal**      | 218 (68%) |
| β-hemolytic       |      |
| Group A           | 32   |
| Group B           | 9    |
| Group C           | 2    |
| Group F           | 1    |
| Group G           | 4    |
| Nongroupable      | 1    |
| **Subtotal**      | 49 (15%) |
| Total             | 321  |

* Not further identified.

(65%), followed by group B (18%), group G (8%), group C (2%), and one isolate of each of group F and a nongroupable strain.

**DISCUSSION**

Streptococcal terminology can be confusing inasmuch as classification may be according to hemolytic reactions in blood (α, β, or γ), biological characteristics (pyogenic, viridans, enterococcus, lactic), or antigenic analysis (Lancefield groups). These categories are not mutually exclusive; for example, a group D streptococcus may be enterococcal or non-enterococcal and may yield an α, β, or γ reaction on blood agar. In many instances, therefore, an organism will be described by more than one means of classification. Viridans
in extrarespiratory streptococcal infections has been reviewed by Reinarz and Sanford (14), Feingold et al. (9), and Duma et al. (6). Of interest in recent years has been the role these organisms have played in causing opportunistic infections (1, 10, 11). Although presumptive identification of group A streptococci may be provided with reasonable accuracy by the selective inhibition of these strains by a 0.02- or 0.04-unit bacitracin disk (15), serological grouping is necessary for definitive identification of group A and other \( \beta \)-hemolytic streptococci.

The taxonomy of some human viridans streptococci was recently reviewed by Colman and Williams (4), who proposed recognition of six species of these organisms: \( \text{S. pneumoniae} \), \( \text{S. salivarius} \), \( \text{S. mitior} \), \( \text{S. milleri} \), \( \text{S. sanguis} \), and \( \text{S. mutans} \). They indicated preference for the term \( \text{S. mitior} \), rather than \( \text{S. mitis} \), for a variety of reasons, including precedence of terminology and poor definition of \( \text{S. mitis} \). Be that as it may, Colman and Williams have emphasized that \( \text{S. salivarius} \), \( \text{S. mitior} \), \( \text{S. milleri} \), \( \text{S. sanguis} \), and \( \text{S. mutans} \) may react with various Lancefield group antisera. By using immunochemical, metabolic, and genetic analysis of viridans streptococci, Austrian et al. (2) found strains that cross-reacted with several pneumococcal capsular serotypes. Many others with capsular antigens not cross-reacting with pneumococci could be classified into one or another of 24 capsular serotypes.

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