Identity of viridans streptococci isolated from cases of infective endocarditis

C. W. I. DOUGLAS, J. HEATH, K. K. HAMPTON* and F. E. PRESTON*

Department of Oral Pathology, School of Clinical Dentistry, University of Sheffield and *Department of Haematology, Royal Hallamshire Hospital, Sheffield

Summary. The oral streptococci have undergone considerable taxonomic revision in recent years but there is still little information concerning associations between the newly defined species and disease. This study examined the identities of 47 strains of oral streptococci collected from 42 confirmed cases of infective endocarditis. By means of recently described physiological schemes, the most common species identified were Streptococcus sanguis sensu stricto (31.9%), S. oralis (29.8%) and S. gordonii (12.7%). Other related species including S. mitis and "S. parasanguis" were less common. This indicates that attention should be focused on S. sanguis sensu stricto and S. oralis when considering possible pathogenic mechanisms involved in viridans streptococcal endocarditis.

Introduction

Oral streptococci are among the most common causes of infective endocarditis, and of these, Streptococcus sanguis, "S. mitior" and S. mutans are isolated most frequently. However, recent taxonomic studies of these organisms have resulted in the recognition of several new species as well as the re-definition of older ones and many of the earlier endocarditis isolates now carry different names. For example, S. sanguis is now divided into S. sanguis sensu stricto and S. gordonii. A streptococcus, closely related to S. sanguis, and characterised by having tufts of surface fibrils, has been named S. crista. "S. mitior" is no longer described as such and has been re-named S. mitis. Strains described previously as "dextran-positive mitior" are now "S. oralis" whilst "S. milleri," previously an ill-defined heterogeneous group of organisms, has been divided among three species—S. anginosus, S. intermedius and S. constellatus. Also, some strains originally thought to belong to the "milleri group", but which could not be classified as any of the above species, have now been grouped into a new species, "S. parasanguis", related to S. sanguis. S. vestibularis is a new species similar to S. salivarius. Finally, the "mutans streptococci" are now divided into seven species.

Although such changes have largely ended the state of taxonomic confusion that has been characteristic of the viridans streptococci for many years, it is important that the association of individual species with disease be re-assessed in relation to recognition of important pathogenic traits.

The aim of the present study was to determine the identities of oral streptococci isolated from cases of infective endocarditis in the light of the new taxonomy.

Materials and methods

Bacterial strains

Forty-seven strains of viridans streptococci were collected from several centres in the UK and from one centre in Germany. These were isolated from 42 confirmed cases of endocarditis, but the clinical information was available for only 17 patients and, therefore, we have no knowledge of patients’ symptoms or previous medical history, such as presence of prosthetic valves and recent dental treatment. Strains were stored freeze-dried.

Identification

Growth from a blood-agar plate was removed with a sterile cotton swab. Half the growth was resuspended in 1 ml of sterile water and the other half in 1 ml of 0.1 M Tris-HCl, pH 7.0. Organisms suspended in water were tested for their ability to ferment raffinose, inulin, N-acetylglucosamine and mannitol, and for hydrolysis of aesculin and arginine. For fermentation reactions, sugars (1% w/v) were included in a basal medium comprising Purple Broth Base (Difco) 16 g/L, Thioglycollate Broth (Difco) 24 g/L and yeast extract (Lab M) 5 g/L, pH 7.2. Broths were incubated in CO₂ at

Received 12 Feb. 1993; accepted 18 Feb. 1993.
Correspondence should be sent to Dr C. W. I. Douglas, Department of Oral Pathology, School of Clinical Dentistry, Claremont Crescent, Sheffield S10 2TA.

179
Table I. Identification scheme for viridans streptococci

<table>
<thead>
<tr>
<th>Test</th>
<th>S.sI</th>
<th>S.sII</th>
<th>S.sIII</th>
<th>S.o.</th>
<th>S.p.</th>
<th>S.g.</th>
<th>S.mi</th>
<th>S.sl</th>
<th>S.mu</th>
<th>S.b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase binding</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aesculin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>arginine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acid from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>raffinose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>V</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>inulin</td>
<td>+</td>
<td>V</td>
<td>V</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N-acetylglucosamine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Enzymes:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sialidase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N-acetylgalactosaminidase</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>β-fucosidase</td>
<td>-</td>
<td>+</td>
<td>V</td>
<td>-</td>
<td>V</td>
<td>-</td>
<td>V</td>
<td>-</td>
<td>V</td>
<td>-</td>
</tr>
<tr>
<td>α-glucosidase</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>α-arabinosidase</td>
<td>-</td>
<td>V</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>V</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N-acetylglucosaminidase</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+, > 80% positive; -, < 15% positive; V, 15-80% positive. (Results for table assembled from references 1, 3, 4, 5, 15).

S.s, S. sanguis biotypes I, II, III; S.o, S. oralis; S.p, "S. parasanguis"; S.g, S. gordonii; S.mi, S. mitis; S.sl, S. salivarius; S.mu, S. mutans; S.b, S. bovis.

37°C and assessed after 24, 48 and 72 h. Aesculin hydrolysis and the production of ammonia from arginine were tested by the method described by Bisset and Davis; the production of ammonia from arginine was detected by the addition of Nessler's reagent.

Organisms suspended in Tris buffer were assessed for the constitutive enzymes N-acetylneuraminidase, β-fucosidase, N-acetylglactosaminidase, α-glucosidase, α-arabinosidase and N-acetylglucosaminidase in tests with the fluorogenic 4-methylumbelliferyl (MU) substrates 2’-4-MU-α-D-N-acetylneuraminic acid, 4-MU-β-D-fucoside, 4-MU-N-acetyl-galactosaminide, 4-MU-α-D-glucoside, (4-MU)-α-L-arabinoside and 4-MU-N-acetyl-glucosaminide respectively (Sigma). Substrates were dissolved in a small volume of dimethyl sulphoxide and diluted for use into 0.1 M Tris-HCl, pH 7.5, to a final concentration of 100 μg/ml.

Ability to bind salivary amylase was assessed by the method described by Douglas. The scheme used for the identification of strains was that described by Beighton et al. supplemented by that of Douglas et al. for amylase-binding discrimination (table I).

Results

The numbers of isolates from endocarditis cases ascribed to each species are shown in table II. The 47 strains were divided among eight species. S. sanguis (15 strains, 32%) and S. oralis (14, 29-8%) were the two most common, followed by S. gordonii (6, 12-7%), S. bovis and the remaining oral species were isolated relatively infrequently. Biotypes 2 and 3 of S. sanguis were more common than biotype 1.

The majority (82%) of strains fitted the identification scheme exactly and the remaining strains were identified to within one or two test reactions. In the latter cases, greater emphasis was put on the results of tests that had reported as 100% reactions for particular species. One strain could not be identified.

Discussion

There have been several studies on the microbial aetiology of infective endocarditis but these neither speciated the viridans streptococci nor were the schemes employed capable of differentiating the new species. Table III shows a summary of the results of five such studies in which S. sanguis and "S. mitior" were consistently the species most frequently isolated. With the exception of the study by Parker and Ball, no information has been given on the physiological characteristics of the strains isolated, and so it is not possible to deduce their likely identities in the light of the new taxonomy. The results of this study differ from previous findings mainly in that S. oralis was found to be a frequent isolate from endocarditis cases and S. mitis and S. mutans were uncommon. However, in line with previous reports, S. sanguis proved to be the numerically dominant species and must, therefore, still be considered the most important of the oral streptococci causing infective endocarditis. The closely related species, S. gordonii, which would have been classified as S. sanguis in previous studies, was the third most prominent group, although it represented less than half the number of S. sanguis sensu stricto isolates. Although it could be said that the isolates studied here represented a biased collection, this is unlikely as approximately half of them came from district hospitals rather than from regional referral centres.

Parker and Ball have given a full description of the strains isolated from endocarditis in their study.
Strains designated “dextran-positive mitior” should now be called S. oralis and it is likely that some of the “S. mitior” strains might also be described as S. oralis, despite giving negative results in tests for dextran, the major differentiating factor used. Kilian et al. reported that 22% of S. oralis strains failed to produce dextran detectable by an alcohol precipitation test; thus, if a similar proportion of “S. mitior” strains in the study of Parker and Ball proved to be dextran-negative S. oralis, this would become an important species in their series. It is probable that S. oralis has been under-reported in previous studies of infective endocarditis.

Because S. sanguis, S. oralis and, possibly, S. gordonii are frequently isolated from cases of infective endocarditis, it is tempting to speculate that they have pathogenic features particularly relevant to the disease. All three species produce extracellular dextran from sucrose and there is evidence of fibronectin when this is adsorbed to a collagen surface, a situation that might be expected to prevail in an area of damaged endothelium. In contrast, little is known about potential pathogenic features of S. oralis that might be relevant to endocarditis, although the species has a significant glycosidase potential, including N-acetyl-neuraminidase activity, and many plasma glycoproteins carry N-acetylneuraminic acid groups. Also, S. sanguis biotypes 2 and 3, as defined by Beighton et al., exhibit more glycolytic activity than type 1 strains, and the former were more frequent isolates in this study than the latter. It may be that S. sanguis and S. oralis grow more successfully in plasma or thrombotic vegetations than other species of oral streptococci.

With improvements in taxonomy of the oral streptococci and the development of rapid physiological schemes for their identification, it is becoming possible to focus attention on pathogenic mechanisms of the organisms that are of greatest importance in infective endocarditis.

This work was funded by the British Heart Foundation, grant number 16/91.

References
10. Whiley RA, Fraser HY, Douglas CWI, Hardie JM, Williams AM, Collins MD. Streptococcus parasanguis sp. nov., an