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Identity of viridans streptococci isolated from cases of infective endocarditis

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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,[1–5] and of these, *Streptococcus sanguis*, "*S. mitior*" and *S. mutans* are isolated most frequently.[1–5] However, recent taxonomic studies of these organisms have resulted in the recognition of several new species as well as the redefinition 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*.[6] A streptococcus, closely related to *S. sanguis*, and characterised by having tufts of surface fibrils, has been named *S. crista*.[7] "*S. mitior*" is no longer described as such and has been re-named *S. mitis*.[6] Strains described previously as "dextran-positive mitior" are now "*S. oralis*"[6,8] whilst "*S. milleri*," previously an ill-defined heterogeneous group of organisms, has been divided among three species—*S. anginosus*, *S. intermedius* and *S. constellatus*.[9] 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*.[10] *S. vestibularis* is a new species similar to *S. salivarius*.[11] Finally, the "mutans streptococci" are now divided into seven species.[12]

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 CO_2 at

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Table I. Identification scheme for viridans streptococci

Test	Results characteristic of species									
	S.sI	S.sII	S.sIII	S.o.	S.p.	S.g.	S.mi	S.sl	S.mu	S.b
Amylase binding	−	−	−	−	+	+	+	±	−	−
Hydrolysis of:										
aesculin	+	+	−	−	−	+	−	+	+	+
arginine	+	+	+	−	+	+	−	−	−	−
Acid from:										
raffinose	+	+	−	+	V	−	+	V	+	+
inulin	+	V	V	−	−	+	V	V	+	+
N-acetylglucosamine	+	+	+	+	+	+	+	+	+	+
mannitol	−	−	−	−	−	−	−	−	+	+
Enzymes:										
sialidase	−	−	−	+	−	−	V	−	−	−
N-acetylgalactosaminidase	−	−	−	+	+	V	−	−	−	V
β-fucosidase	−	+	V	−	V	−	−	V	−	V
α-glucosidase	−	−	−	+	+	V	+	V	+	V
α-arabinosidase	−	−	−	−	V	−	−	+	−	−
N-acetylglucosaminidase	−	V	+	+	+	+	−	−	−	+

+, > 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;[13] 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-acetylgalactosaminidase, 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.[14] The scheme used for the identification of strains was that described by Beighton *et al.*[15] supplemented by that of Douglas *et al.*[16] 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 been reported as 100% reactions for particular species.[6,15,16] 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,[17] 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.[17]

Table II. Identities of streptococcal isolates

Species	Number	(%) of isolates
S. sanguis biotype 1	3	(6·4)
S. sanguis biotype 2	6 } 15	(12·7) } (31·9)
S. sanguis biotype 3	6	(10·6)
S. oralis	14	(29·8)
S. gordonii	6	(12·7)
S. bovis	3	(6·4)
"*S. parasanguis*"	2	(4·2)
S. mitis	2	(4·2)
S. mutans	2	(4·2)
S. salivarius	2	(4·2)
Unidentified	1	(2·1)

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.*[6] reported that 22% of *S. oralis* strains failed to produce dextran detectable by an alcohol precipitation test;[18] 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 to suggest that dextran promotes adhesion to thrombus in the rabbit endocarditis model.[19] Also, it has been shown that *S. sanguis* strains are able to aggregate human platelets *in vitro* more effectively than other species;[20] this may enable these organisms to contribute to thrombus formation on endothelial surfaces.[21] Studies on one strain of *S. gordonii* have shown that it can attach to fibronectin when this is adsorbed to a collagen surface,[22] 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.*,[15] 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.

Table III. Reports on the microbial aetiology of infective endocarditis (percentages of isolates)

Organism	Bayliss *et al.*[1]	Roberts *et al.*[2]	Young[3]	Manford *et al.*[4]	Parker and Ball[17]
Streptococci					
β-haemolytic	1	1	5	—	5
S. sanguis	5	24	21	23	17
"*S. mitior*"	5	31	19	13	21
S. mutans	2	7	8	3	14
"*S. miller*"	2	4	5	3	5
S. salivarius	1	1	4	—	1
S. bovis	5	27	—	—	17
NVS*	—	5	—	—	—
S. faecalis	2	7	5	6	8
α-haemolytic (not identified)	33	1	43	10	11
Other organisms	27	39	34	25	—
Culture negative	9	3	—	5	—

* NVS, nutritionally variant streptococci.

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