

Streptococcus intermedius, *Streptococcus constellatus*, and *Streptococcus anginosus* (the *Streptococcus milleri* group): Association with Different Body Sites and Clinical Infections

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The associations of *Streptococcus intermedius*, *S. constellatus*, and *S. anginosus* (the three species of the *S. milleri* group) with clinical infections and sites of isolation were investigated by using a simple biochemical scheme to identify a collection of 153 clinical isolates. *S. intermedius* was associated with abscesses of the brain and liver, while both *S. anginosus* and *S. constellatus* were isolated from a wider range of sites and infections. *S. anginosus* strains predominated in both genitourinary and gastrointestinal sources and exhibited a wider range of phenotypes, particularly in the ability to ferment mannitol and/or raffinose.

Streptococcus intermedius, *S. constellatus*, and *S. anginosus* (collectively referred to as the *S. milleri* group) form part of the normal flora of the mouth, gastrointestinal tract, and genitourinary tract and are often associated with purulent infections (5, 12).

Despite increasing awareness of their clinical significance, the classification, nomenclature, and identification of the *S. milleri* group has, until recently, remained confused (4), preventing demonstration of clear associations between particular species and specific sites of isolation and disease conditions. However, recent genetic and phenotypic studies in our laboratories have shown that the *S. milleri* group consists of three distinct species, *S. intermedius*, *S. constellatus*, and *S. anginosus*, that can be differentiated phenotypically from each other and from other viridans group streptococci (1) by a short scheme of biochemical tests (9–11). Consequently, we were able to identify and characterize a large number of these bacteria isolated from a wide variety of clinical sources, and this enabled us to determine associations between types of infection and particular species.

We obtained 153 strains of the *S. milleri* group from different laboratories in the United Kingdom and abroad. These included 18 strains from head and neck sites; 21 from the central nervous system (CNS); 20 from brain abscesses; 1 from cerebrospinal fluid; 12 from respiratory tracts; 24 from gastrointestinal tracts; 9 from other abdominal or pelvic sites; 44 from genitourinary sources; 19 from skin, soft tissues, and bone; and 4 from blood cultures (unspecified clinical backgrounds).

All strains were tested for production of α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, β -*N*-acetylglucosaminidase, β -*N*-acetylglucosaminidase, sialidase, and β -*D*-fucosidase by using 4-methylumbelliferyl-linked fluorogenic substrates in microtitration trays as described previously (10). Acid production from the carbohydrates amygdalin, arbutin, inulin, lactose, mannitol, melibiose, *N*-acetylglucosamine, sorbitol, and raffinose and tests for hydrolysis of esculin and arginine were performed in microti-

tration trays by the method of Beighton et al. (2). Hyaluronidase production was tested for by the rapid plate method of Smith and Willett (8). The hemolytic reactions were determined on Columbia agar (GIBCO Europe Ltd., Paisley, Scotland) containing 5% (vol/vol) defibrinated horse blood. Lancefield grouping was performed with the Streptococcal Grouping Kit (UNIPATH Ltd., Basingstoke, Hants, England), in accordance with the manufacturer's instructions, on those strains received without accompanying serological data. Assignment of strains to either *S. anginosus*, *S. constellatus*, or *S. intermedius* was done on the basis of the identification scheme of Whiley et al. (10).

Table 1 shows the numbers of strains of the three species identified in each of the broad clinical categories. The results demonstrate a marked association of *S. intermedius* with infections involving the CNS, although this species, previously shown to be the species of the *S. milleri* group most commonly isolated from liver abscesses and dental plaque (10), exhibited an otherwise relatively limited clinical distribution, being identified infrequently from respiratory and gastrointestinal tracts and not at all from genitourinary sources. Conversely, *S. anginosus* was noticeably the most frequently identified of the three species from gastrointestinal tracts, as well as from genitourinary sources, and was identified commonly from most of the other broad clinical categories, except for the CNS. *S. constellatus* was also frequently identified among strains from most of the clinical categories, except for the CNS, but did not predominate in any particular broad clinical category, with the possible exception of the respiratory tract.

S. intermedius and *S. constellatus* strains exhibited the same relatively homogeneous set of biochemical test reactions as we have reported previously (10). However, we found a greater diversity of fermentation reactions among the strains of *S. anginosus* studied here than we had previously found. All strains of the *S. milleri* group, except one, which was identified as an *S. intermedius* strain, that fermented either raffinose or mannitol or both were identified as *S. anginosus* strains (54 strains fermented both sugars, 1 fermented mannitol only, and 6 fermented raffinose only). The distribution of these broadly fermentative *S. anginosus*

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TABLE 1. Association of *S. anginosus*, *S. constellatus*, and *S. intermedius* with clinical sources

Clinical source(s)	No. of strains identified as:			Total no. of strains
	<i>S. anginosus</i>	<i>S. constellatus</i>	<i>S. intermedius</i>	
Head and neck ^a	8 (44) ^b	7 (39)	3 (17)	18
CNS ^a	2 (10)	1 (5)	18 (86)	21
Respiratory tract	4 (29)	7 (57)	1 (14)	12
Gastrointestinal tract	19 (79)	4 (17)	1 (4)	24
Other abdominal or pelvic sites	4 (44)	3 (33)	2 (22)	9
Genitourinary tract	40 (91)	4 (9)	0 (0)	44
Skin, soft tissues, bone	10 (53)	5 (26)	4 (21)	19
Blood	2 (50)	2 (50)	0 (0)	4
Total no. of strains of each species identified	89	33	29	151

^a One strain remained unidentified in each of these categories.

^b Percentages of the three species from each main clinical source are shown in parenthesis.

strains was of interest, as 35 (88%) of 40 strains from genitourinary sources fermented one or both sugars, as did 10 (53%) of 19 strains from gastrointestinal tracts, 2 (50%) of 4 strains from other abdominal and pelvic sites, and 7 (70%) of 10 strains from skin, soft tissue, or bone. This contrasts with the absence of these broadly fermentative strains among *S. anginosus* isolates from the head and neck, CNS, and respiratory tract. Within the gastrointestinal tract, these broadly fermentative strains formed the majority of *S. anginosus* strains, i.e., 9 (64%) of 14 strains from the rectal mucosa of patients with colitis and 2 (100%) of 2 strains from perianal sources, but were absent among *S. anginosus* strains from the upper gastrointestinal tract.

The biochemical characteristics of the three species did not differ markedly from the data previously reported in our preliminary study of 157 strains (including 58 from normal dental plaque) (10). The increased proportion of *S. anginosus* strains which produced β -galactosidase and fermented mannitol and/or raffinose was largely due to the inclusion in this study of 40 isolates from genitourinary sources.

Earlier investigations into the associations between clinical distribution and particular species or subgroups within the *S. milleri* group have been hampered by the inability of investigators to recognize these three species because of frequent use of characteristics which are unreliable for identification of *S. intermedius*, *S. anginosus*, and *S. constellatus* (10), namely, possession of a Lancefield group antigen, the type of hemolysis produced, and the ability to ferment lactose (3, 6, 7). With this study, the increase in the total number of strains in the *S. milleri* group examined from clinical sources since our previous report (10) has more clearly demonstrated associations of these species with particular types of clinical conditions and provides a stimulating basis for future investigations into the possible routes of entry of these bacteria, their ability to colonize particular anatomical sites, and potential virulence factors.

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