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Streptococcus salivarius Meningitis Case Strain Traced to Oral Flora of Anesthesiologist[▽]

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Two women in labor received intrapartum spinal anesthesia from the same anesthesiologist approximately 1 h apart. Within 15 h, both patients developed *Streptococcus salivarius* meningitis and one patient died. Blood and cerebrospinal fluid (CSF) samples from both patients and tongue swab specimens from the anesthesiologist yielded isolates of an indistinguishable *S. salivarius* strain.

Streptococcus salivarius is commonly found among normal oral flora, where it is the predominant species cultivated from tongue dorsa (13), and has been used as a reliable marker for forensic identification of saliva using DNA amplification techniques (14). Recent multilocus-sequence-based investigations have indicated that, similar to other streptococcal species (1, 7, 8), *S. salivarius* is a distinct species that displays high genetic diversity and undergoes a high level of genetic exchange (3, 6).

Of 75 cases of meningitis occurring after lumbar puncture recorded from 1952 to 1998, all 56 cases where bacterial classification was provided were due to streptococcal species, with *S. salivarius* the most common species identified (2). Independent cases of *S. salivarius* meningitis circumstantially linked to the same anesthesiologist have previously been reported (15, 17).

Recently, 2 women in Ohio developed *S. salivarius* meningitis shortly after receiving intrapartum spinal anesthesia (4). Initial investigation revealed that the blood and cerebrospinal fluid (CSF) isolates from the two patients displayed identical chromosomal restriction digest patterns resolved by pulsed-field gel electrophoresis (PFGE). Putative *S. salivarius* was identified within oral and saliva specimens of the anesthesiologist (taken 2 days after the anesthesia had been administered to the patients) using previously described PCR assays for this species (12, 14). Initial attempts to isolate *S. salivarius* from the anesthesiologist carriage specimens were not successful, possibly in part because the anesthesiologist had received ciprofloxacin for meningococcal prophylaxis within 12 h of the onsets of symptoms in the 2 patients. Here, we report additional data on this investigation, including the successful isolation from these specimens of a strain of *S. salivarius* that was genetically indistinguishable from the case strain.

The isolates from the 2 meningitis cases were identified as *S. salivarius* by a conventional biochemical identification scheme (9) and the rapid ID32 STREP method (bioMérieux, Inc.) as described by the manufacturer (10). The isolates were urease positive and had identical biochemical patterns. The rapid ID32 STREP method also displayed identical profiles, with 99.9% identity to the *S. salivarius* standard profile in the manufacturer's database. These isolates were atypical with regard to most reference strains of *S. salivarius* isolates because of their ability to acidify sorbitol (9).

Dorsal tongue, buccal, and nasopharyngeal (NP) swabs taken from the anesthesiologist 2 days after administration of anesthesia were shipped to the CDC *Streptococcus* Laboratory in Amies transport medium. Swabs were placed in 1 ml Todd-Hewitt (TH) broth and vortexed, and 100 μl of the suspension was used to prepare serial dilutions on Trypticase soy agar plates containing 5% sheep blood (BAPs) and colistin nalidixic acid (CNA) plates. The plates were incubated for 24 h at 35°C to obtain isolated colonies. The remaining broth (900 μl) was incubated overnight at 35°C and stored at 4°C for subsequent use. The remaining serial dilutions were also stored at 4°C for subsequent use.

All specimens except for the NP swab yielded a mixture of colony types, with many alpha-hemolytic colonies evident. The NP swab yielded only Gram-negative rods upon subculture on BAP and no growth on CNA agar, potentially as a consequence of ciprofloxacin prophylaxis. Since the patients' *S. salivarius* isolates were urease positive, urea slants were used to screen suspected alpha-hemolytic colonies isolated from the anesthesiologist's cultures. Approximately 300 alpha-hemolytic colonies from CNA plates were found to be urease negative and thus differed from the meningitis patient case isolates. After depletion of the urea slants on hand, an additional 47 colonies were tested using the rapid ID32 STREP method. None of the isolates were identified as *S. salivarius*, based on the manufacturer's profile, and the majority were identified as members of the *Streptococcus anginosus* group. Original swabs were stored at −70°C in glycerol broth for additional testing of isolates once more medium was procured.

Due to the poor results obtained with BAP and CNA media, we procured a more selective medium, Mitis Salivarius agar with 1% potassium tellurite (MSA), which we prepared as described by the manufacturer (Becton Dickinson). MSA con-

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TABLE 1. Multilocus and 16S rRNA gene sequences depicting identical results from meningitis-associated *S. salivarius* isolates from 2 case patients and carriage isolates recovered from their anesthesiologist

Isolate	Allele for indicated gene[a]															
	glcK[b]	*ddlA*[b]	*pepO*[b]	*ilvC*[b]	*thrS*[b]	*pyrE*[b]	*dnaE*[b]	*sodA*[b]	*rpoB*[c]	*sodA*[c]	*pyk*[c]	*ppaC*[c]	*tuf*[c]	*pfl*[c]	*map*[c]	16S rRNA gene
0735[d]	4	Unique	3	4	14	12	Unique	14	Unique	Ref 3	Ref 3	Ref 3	Unique	Unique	Unique	Unique
JIM8221[e]	4	3	3	4	14	12	17	14	ND	ND	ND	ND	ND	ND	ND	ND
SS908	2	ND	ND	ND	ND	ND	ND	Unique	ND	ND	ND	ND	ND	ND	ND	AF459433[f]

[a] "Unique" indicates that the sequences for the genes (the *ddlA*, *dnaE*, *sodA*, *rpoB*, *tuf*, *pfl*, *map*, and 16S rRNA genes) were unique to these case and carriage isolates (corresponding to GenBank accession numbers GU556184, Gu556185, GUU556186, GU556187, GU556190, GU556191, GU556192, and GU175444, respectively). "Ref 3" indicates sequences identical to that given for the allele (in the database at http://viridans.eMLSA.net/) that is from *S. salivarius* strain sk729 (also described in reference 3); the other 5 targets in this MLST scheme for strain sk729 differed from those observed for 0735. ND, not determined.

[b] These targets are as described in reference 6. Results with simple numbers indicate sequence identity to alleles with the same designation in this reference.

[c] These targets are as described in reference 3 and at http://viridans.eMLSA.net/.

[d] Identical allelic and 16S rRNA gene sequences were obtained for brain isolate 0735, 4 additional CSF or blood isolates from the 2 case patients, and 12 carriage isolates recovered from the anesthesiologist.

[e] Results for this isolate were obtained from an oral carriage strain highly related to 0735, taken from reference 6.

[f] GenBank accession number for the 16S rRNA sequence found within reference strain SS908.

tains the selective agents potassium tellurite and crystal violet, which allow for the isolation of the *Streptococcus mitis* group, *S. salivarius*, and enterococci from grossly contaminated cultures (5). Trypan blue in the agar gives these bacteria a distinctive blue color, and *S. salivarius* strains produce a distinctive blue "gum drop" colony. The frozen swabs corresponding to the anesthesiologist's dorsal tongue specimens were placed in 1 ml TH broth, incubated for 4 h at 35°C, and subcultured on MSA in the same manner as described above for BAPs and CNA plates. Several relatively large blue, opaque, mucoid colonies 2 to 4 mm in diameter shared identical colony morphology on this medium with the meningitis case *S. salivarius* isolates. Subsequent conventional biochemical and rapid ID32 STREP testing of cultures from 12 of these colonies revealed a profile identical to those observed for the case blood and CSF isolates. One additional strain of *S. salivarius* that was different from these was also isolated. Colony counts revealed that fewer than 1% of alpha-hemolytic colonies had the distinctive mucoid phenotype of *S. salivarius*. This low percentage is probably due to the anesthesiologist's prophylaxis prior to the collection of carriage specimens, but the presence of some colonies indicates that prophylaxis was not sufficient to eradicate the strain.

PFGE of chromosomal digests and 16S rRNA gene sequencing were performed as previously described (4, 16). The same PFGE pattern and 1493 base 16S rRNA sequence was shared between the case isolates and 12 independent carriage isolates from the anesthesiologist. This 16S rRNA sequence revealed 1 to 3 base substitutions compared to the closest known sequences in GenBank, both identified as *S. salivarius* 16S rRNA genes (accession numbers AF459433 and AY188352). The GenBank accession numbers for the 16S rRNA sequence and other unique DNA sequences identified from this *S. salivarius* strain represented by the case and carriage isolates described here are listed in Table 1.

Viridans streptococci, of which *S. salivarius* is a member species, have been difficult to speciate, potentially due to recombination among closely related species, the technical difficulties of phenotypic analysis, and the close similarities shared between 16S rRNA genes. The website http://viridans.emlsa.net/ offers a system based upon concatenated sequences of seven chromosomally unlinked housekeeping genes that can be used for constructing trees that contain known species-specific clusters (3). We employed the DNA primers at this site (http://www.emlsa.net/#instructions) to amplify and determine the corresponding sequences of the 7 housekeeping gene fragments from 5 meningitis case isolates (blood and CSF isolates from the 2 patients and a brain isolate from the deceased patient) and 12 representative carriage isolates. These 7 sequences obtained from these 17 isolates shared complete identity. Using the software of the site, we found that the 7 concatenated sequences from this meningitis/carriage strain, here designated 0735, clustered closely together in a single cluster within the 4 *S. salivarius* species strains included at the site and distinct from 19 other species included within the mitis, anginosus, and salivarius group streptococci (data not shown; see http://viridans.emlsa.net/example/default.php to view the complete species-resolving tree based upon the current GenBank database). The concatenated query sequence of 3,063 bp from strain 0735 shared 97.13% to 99.41% identity with the other 4 *S. salivarius* strains, with the next best match of 94.97% identity to *Streptococcus vestibularis* (one of the 3 species, including *S. salivarius*, that are recognized in the salivarius group [9]).

In addition, these isolates shared sequence identity between all 8 targets that were used in a second recently described multilocus sequence typing (MLST) scheme for the *S. salivarius* group (6). It was interesting to note that the allelic pattern from 0735 was divergent (sharing 0 to 2 alleles) with 26 of 27 *S. salivarius* strains described in this previous report (6). Interestingly, 0735 shared high relatedness (6 of 8 identical alleles) with a single carriage isolate (JIM8221) described in this study (6), differing only within 2 loci that were unique to 0735. This corresponds to the observation that the 27 *S. salivarius* strains described in the previous MLST study (6) could not be resolved into carriage and infection-associated sets using phylogenetic analysis. The high degree of genetic diversity apparent in this species is also evident from the 3 targets sequenced from the CDC *S. salivarius* reference strain SS908. The 2-gene MLST allelic profile (Table 1) (*glcK* and *sodA*) differed from those observed for the 27 isolates previously described in this MLST scheme (6), although its 16S rRNA sequence shared identity with a previously described *S. salivarius* isolate (GenBank accession number AF459433).

In summary, the sterile site and anesthesiologist carriage *S.*

salivarius isolates described here were genetically indistinguishable from each other with the use of PFGE and two independent MLST schemes. These included invasive (from CSF, blood, and brain) isolates recovered from the 2 meningitis case patients and dorsal tongue isolates from the anesthesiologist who performed spinal anesthesia shortly before the case patients developed symptoms of meningitis. The unique MLST-based profiles and 16S rRNA sequence of the putative causal strain compared with corresponding genetic profiles of all 31 *S. salivarius* strains previously genotyped using MLST (3, 6) provide compelling circumstantial evidence that the source of the two meningitis infections was a specific *S. salivarius* strain present in the normal oral flora of the anesthesiologist. We are aware of one similar instance where a meningitis isolate of *S. salivarius* shared identical genotype and fatty acid profiles with a carriage isolate from the operating neurologist (17). We emphasize that in this specific situation, the commercial selective medium (Mitis Salivarius agar) was required to recover the carriage isolates of the *S. salivarius* strain described here. We believe that the situation described here supplements cumulative data that should mandate the usage of surgical masks when lumbar punctures are performed (11, 18).

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