Streptococcus salivarius Meningitis Case Strain Traced to Oral Flora of Anesthesiologist

Patricia L. Shewmaker,¹ Robert E. Gertz, Jr.,¹ Clara Y. Kim,²,³ Sietske de Fijter,³ Mary DiOrio,³ Matthew R. Moore,¹ and Bernard W. Beall¹*

Respiratory Diseases Branch, Division of Bacterial Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia¹; Epidemic Intelligence Service Program, Office of Workforce and Career Development, Centers for Disease Control and Prevention, Atlanta, Georgia²; and Ohio Department of Health, Columbus, Ohio³

Received 1 March 2010/Returned for modification 30 April 2010/Accepted 17 May 2010

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 onset 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-
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

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Allele for indicated genea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>glk c&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0735d</td>
<td>4</td>
</tr>
<tr>
<td>JIM8221&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4</td>
</tr>
<tr>
<td>SS908</td>
<td>2</td>
</tr>
</tbody>
</table>

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, GU556186, GU556187, GU556190, GU556191, GU556192, and GU175444, respectively).

b 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.

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

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

f Identical allele 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.

PMGs of chromosomal digests and 16S rRNA gene sequencing were performed as previously described (4, 16). The same PMG 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 speculate, 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 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) (glk 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).
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 (Mititis 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).

We are grateful to Fabiana Pimenta, who, on a weekend, coordinated shipments of isolates and specimens related to this study to the Streptococcus Laboratory.

REFERENCES