Streptococcus galloyticus Subspecies pasteurianus (Biotype II/2), a Newly Reported Cause of Adult Meningitis

Amy S. Sturt, Liying Yang, Kuldip Sandhu, Zhiheng Pei, Nicholas Cassai, and Martin J. Blaser

Stanford University, Stanford, California; New York University, New York, New York; and New York Veterans Affairs Healthcare System, New York, New York

Received 14 January 2010/Returned for modification 27 January 2010/Accepted 26 March 2010

We report the first case of adult meningitis confirmed to be due to Streptococcus galloyticus subsp. pasteurianus. Phenotypically reported as Streptococcus bovis biotype II/2, 16S rRNA sequencing revealed S. galloyticus subsp. pasteurianus. Because of taxonomic uncertainties, S. galloyticus subsp. pasteurianus may be an under-recognized agent of systemic infections.

The group D nonenterococcal streptococci include Streptococcus bovis, with two biotypes (I and II) that cause human infections. Biotype I (Streptococcus galloyticus) is associated with colon cancer and endocarditis (20). Biotype II/1 (Streptococcus infantarius) has been associated with noncolonic cancers (5). These clinical implications make accurate species identification critical. However, the S. bovis group is genetically diverse, and organisms previously classified as S. bovis now represent multiple species with unique clinical manifestations (8, 9, 22). S. galloyticus subsp. pasteurianus, also named Streptococcus pasteurianus, was proposed to replace S. bovis II/2 (19, 22). Clinicians and laboratory staff do not recognize this taxonomy and its associated clinical implications. We report a case of S. galloyticus subsp. pasteurianus meningitis.

A 75-year-old man presented to the emergency room 2 days after the onset of headache, fever, and photophobia. He had a history of prostate cancer 8 years previously, which was treated with pelvic irradiation, with subsequent radiation proctitis. He denied intravenous drug abuse. Physical exam revealed a temperature of 38.3°C, photophobia, and nuchal rigidity. His peripheral white blood cell count (WBC) was 11,400/mm3 (with 65% neutrophils, 15% bands, and 10% lymphocytes), and his glucose was 160 mg/dl. The patient was given 1 g ceftriaxone, clindamycin, erythromycin, levofloxacin, linezolid, penicillin, and vancomycin, both ampicillin and vancomycin were discontinued. A transesophageal echocardiogram showed no evidence of endocarditis, and colonoscopy was negative. He received intravenous antibiotics for 10 days, and as of January 2010 has not had recurrence of illness after 54 months of follow-up.

After incubation on tryptic soy blood agar (TSBA) plates, colonies were tested for catalase production and failed growth in 6.5% NaCl. Lancefield typing was determined by using Streptex (Remel). Carbohydrate fermentation analysis was performed using the API 20 Strep (ID 7650450; bioMérieux) and RapID Strep (ID 22301; Remel) kits. See Table 1 for the results of phenotypic testing.

Clinical isolates were cultured on TSBA plates and harvested in 0.5 ml of phosphate-buffered saline, and bacterial genomic DNA was prepared with a DNaseasy tissue kit (Qiagen, Valencia, CA). 16S rRNA genes were amplified from extracted DNA using the primer pair 8F and 1510R, as described previously (18). Using a PCR purification kit (Qiagen), PCR products were purified and ligated with the pGEM-T Easy vector (Promega, Madison, WI) and transformed with Escherichia coli DH5α competent cells. Transformed cells were used as PCR template vector primers. From colonies showing the expected product, inserts were sequenced using primers 8F and 9324 (CSF), one and two clones, respectively, were examined. Phred quality scores and visual inspection were used to determine sequence accuracy.

Sequences were aligned with NAST at Greengenes (http://greengenes.lbl.gov/cgi-bin/nph-index.cgi) (6). Misalignments were manually curated with Molecular Evolutionary Genetics Analysis 3.1 (MEGA 3.1) (14). The phylogenetic tree was generated using MEGA 3.1. Evolutionary distances were calculated with the Jukes-Cantor algorithm (13). The statistical strength of the neighbor-joining method was assessed by bootstrap resampling (500 replicates) (21).

Culture plates with growth of the isolate were layered with 3% phosphate-buffered glutaraldehyde and fixed for 12 h. Postfixation, specimens were embedded in Embed 812 in...
In 1995, Osawa suggested a new species, *S. gallolyticus*, for those organisms able to decarboxylate gallic acid (16). Subsequently, whole-cell protein analysis was used to show that the *S. galloyticus* species comprised *S. bovis* biotypes I and II/2 (7). Later sequencing of *sodA* and DNA-DNA hybridization confirmed the need for the taxonomic change (19, 22). Based on biochemical traits, DNA-DNA relatedness, and 16S rRNA sequences, Schlegel et al. suggested that the *S. galloyticus* species includes three subspecies: *S. galloyticus* subsp. *galloyticus*, *S. galloyticus* subsp. *pasteurianus*, and *S. galloyticus* subsp. *macedonicus* (22). These studies suggest *S. galloyticus* subsp. *pasteurianus* is the preferred nomenclature over *S. pasteurianus*.

The uncertainties in taxonomy cloud the reporting of the accurate spectrum of clinical disease caused by *S. galloyticus* subsp. *pasteurianus*. The organism causes meningitis, bacteremia, peritonitis, and chorioamnionitis in adults (1, 2, 10, 23). Thus far, however, there is not enough information to imply a relationship of adult *S. galloyticus* subsp. *pasteurianus* infection with endocarditis or colonic carcinoma. A recent report associated 63% of 11 bacteremic events with hepatobiliary disease (2). In infants, *S. galloyticus* subsp. *pasteurianus* infection may present as sepsis or meningitis (3, 11, 12, 15).

Findings from reported cases of meningitis due to *S. bovis* biotype II/2 (*S. galloyticus* subsp. *pasteurianus*) in both adults and infants are reported in Table 2. These cases may be underreported in the literature due to taxonomic misidentification. These cases also suggest that *S. galloyticus* subsp. *pasteurianus* infects both full-term and preterm neonates in both early and late onset patterns. From our review, adults with a history of chronic steroid use or compromised gastrointestinal tract integrity may be at risk for meningitis. More research is needed to establish definitive epidemiologic patterns.

This is the first adult meningitis case of *S. galloyticus* subsp. *pasteurianus* to be confirmed by 16S rRNA sequencing. Our patient’s portal of entry may be related to radiation proctitis. The organism’s capsule may explain its central nervous system tropism. The percentage of 21 *S. galloyticus* subsp. *pasteurianus* strains that exhibited the corresponding phenotypic trait (22).
pism. Given the relationship of S. bovis infection with carcinoma, 16S rRNA sequencing should be done on systemic S. bovis isolates until genotypic analysis, nomenclature, and clinical approaches are integrated. We suspect that many of the S. bovis biotype II/2 clinical isolates reported previously may actually represent S. gallolyticus subsp. pasteurianus.

Nucleotide sequence accession numbers. The sequences reported in this paper have been deposited with GenBank and assigned accession numbers EF670541, EF670542, and EF670543.

This study was supported in part by NIH grant R01AI063477 from the National Institute of Allergy and Infectious Diseases and by the Diane Belfer Program in Human Microbial Ecology.

### REFERENCES


### TABLE 2. Reported meningitis cases caused by S. bovis biotype II/2 (S. gallolyticus subsp. pasteurianus)

<table>
<thead>
<tr>
<th>Yr of report</th>
<th>Patient age</th>
<th>Gender</th>
<th>CSF Gram stain</th>
<th>Positive cultures</th>
<th>Antibiotic susceptibility*</th>
<th>Length of antibiotic therapy (days)</th>
<th>Additional clinical information</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993 (10)</td>
<td>61 yrs</td>
<td>Male</td>
<td>Negative</td>
<td>Blood, CSF</td>
<td>Penicillin, cefotaxime*</td>
<td>Not reported</td>
<td></td>
<td>Survived</td>
</tr>
<tr>
<td>2000 (3)</td>
<td>4 wks</td>
<td>Male</td>
<td>Positive</td>
<td>Blood, CSF</td>
<td>Penicillin*</td>
<td>18</td>
<td>Premature delivery</td>
<td>Survived</td>
</tr>
<tr>
<td>2003 (12)</td>
<td>3 days</td>
<td>Male</td>
<td>Positive</td>
<td>Blood, CSF</td>
<td>Penicillin*</td>
<td>14</td>
<td>Not applicable</td>
<td>Survived</td>
</tr>
<tr>
<td>2009 (15)</td>
<td>5 days</td>
<td>Female</td>
<td>Not reported</td>
<td>Blood, CSF</td>
<td>Penicillin, cefotaxime, *imipenem</td>
<td>14</td>
<td>Not applicable</td>
<td>Survived</td>
</tr>
<tr>
<td>Present study</td>
<td>75 yrs</td>
<td>Male</td>
<td>Negative</td>
<td>Blood, CSF</td>
<td>Penicillin, ceftriaxone,*clindamycin, erythromycin, levofloxacin, linezolid, vancomycin</td>
<td>10</td>
<td>Radiation proctitis</td>
<td>Survived</td>
</tr>
</tbody>
</table>

* *: antibiotic chosen for ultimate patient treatment based on results of culture and susceptibility testing.