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

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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 colonic carcinoma 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 had a history of diabetes. He was admitted with peripheral white blood cell count (WBC) of 11,400/mm³ (with 65% neutrophils, 15% bands, and 10% lymphocytes) and a temperature of 38.3°C, photophobia, and nuchal rigidity. His peripheral white blood cell count 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 DNeasy 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 8R. The primers 8F and 8R were used to sequence the region of the 16S rRNA gene containing the variable region. The 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).

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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
firmed the need for the taxonomic change (19, 22). Based on Later sequencing of \( sodA \) data indicated the isolate represents /H11349 S. gallolyticus consequently, whole-cell protein analysis was used to show that the those organisms able to decarboxylate gallic acid (16). Subse-

product should be sufficient for routine clinical purposes. clonal to provide certainty. However, sequencing of the PCR confirmed that the strain could have been identified without forms to the phenotype previously described (Table 1) and identical 16S rRNA genes have not been reported in different S. pasteurianus rRNA genes are identical to the neity (4).

rRNA genes, likely representing true intragenomic heteroge-
neity (4).

Acidification of: Body, MA). Examined using a JEM 1010 electron microscope (JEOL, Pea-
m Epon sections were stained with /H9262 Beem capsules, and 0.07-

Acidification of: 

TABLE 1. Phenotypic characteristics of S. bovis biotype II/2 (S. gallolyticus subsp. pasteurianus)

<table>
<thead>
<tr>
<th>Test</th>
<th>Result for the study patient</th>
<th>% of S. gallolyticus subsp. pasteurianus strains with trait*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>−</td>
<td>0</td>
</tr>
<tr>
<td>Esculin</td>
<td>+</td>
<td>100</td>
</tr>
<tr>
<td>Gallate (tannase activity)</td>
<td>NR</td>
<td>0</td>
</tr>
</tbody>
</table>

Production of:

<table>
<thead>
<tr>
<th>Test</th>
<th>Result for the study patient</th>
<th>% of S. gallolyticus subsp. pasteurianus strains with trait*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetoin</td>
<td>−</td>
<td>100</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>NR</td>
<td>100</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>NR</td>
<td>100</td>
</tr>
<tr>
<td>α-Galactosidase</td>
<td>+</td>
<td>71</td>
</tr>
<tr>
<td>β-Galactosidase (β-Gal)</td>
<td>+</td>
<td>95</td>
</tr>
<tr>
<td>β-Mannosidase</td>
<td>NR</td>
<td>100</td>
</tr>
<tr>
<td>Pyrrolidonyl arylamidase</td>
<td>−</td>
<td>0</td>
</tr>
</tbody>
</table>

Acidification of: Glycogen | − | NR | 0 |
| Inulin                | − | NR | 0 |
| Lactose               | − | NR | 100 |
| Mannitol              | − | NR | 0 |
| Mellibiose            | NR | NR | 10 |
| Raffinose             | + | +  | 57 |
| Starch                | − | NR | 14 |
| Trehalose             | + | NR | 100 |

* The percentage of 21 S. gallolyticus subsp. pasteurianus strains that exhibited the corresponding phenotypic trait (22).

b NR, not reported.

Beem capsules, and 0.07-μm Epon sections were stained with uranyl acetate and lead citrate as previously described (17) and examined using a JEM 1010 electron microscope (JEOL, Pea-
body, MA).

Electron microscopy revealed an encapsulated organism. The 16S sequences for the 2274 clone and one of the two 9324 clones showed 100% sequence identity with the S. pasteurianus type strain CIP105070 (accession number AJ297216) (Fig. 1) (22). Clone 2 from strain 9324 is most closely related to the type strain CIP105070 (accession number AJ297216) (Fig. 1) (22). These studies suggest S. gallo-

lolyticus 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. gallolyticus subsp. pasteurianus. The organism causes meningitis, bactere-

mia, peritonitis, and chorioamnionitis in adults (1, 2, 10, 23). Thus far, however, there is not enough information to impli-
cate a relationship of adult S. gallolyticus subsp. pasteurianus infection with endocarditis or colonic carcinoma. A recent report associated 63% of 11 bacteremic events with hepatopo-
biliary disease (2). In infants, S. gallolyticus 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. gallo-
lolyticus subsp. pasteurianus) in both adults and infants are reported in Table 2. These cases may be underreported in the literature due to taxonomic misidentifica-
tion. These cases also suggest that S. gallolyticus subsp. pas-
teurianus 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 gastroin-
testinal tract integrity may be at risk for meningitis. More research is needed to establish definitive epidemiolog-

ic patterns.

This is the first adult meningitis case of S. gallo-
lolyticus subsp. pasteurianus to be confirmed by rRNA sequencing. Our pa-

tient’s portal of entry may be related to radiation proctitis. The organism’s capsule may explain its central nervous system tro-

biochemical traits, DNA-DNA relatedness, and 16S rRNA se-

quences, Schlegel et al. suggested that the S. gallolyticus species includes three subspecies: S. gallo-
lolyticus subsp. gallo-
lolyticus, S. gallolyticus subsp. pasteurianus, and S. gallolyticus subsp. mace-
donicus (22). These studies suggest S. gallolyticus subsp. past-
teurianus is the preferred nomenclature over S. pasteurianus.

In 1995, Osawa suggested a new species, S. gallo-
lolyticus, for those organisms able to decarboxylate gallic acid (16). Subse-

quently, whole-cell protein analysis was used to show that the S. gallo-
lolyticus species comprised S. bovis biotypes I and II/2 (7). Later sequencing of sodA and DNA-DNA hybridization con-

firmed the need for the taxonomic change (19, 22). Based on
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.

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<table>
<thead>
<tr>
<th>Yr of report (reference)</th>
<th>Patient age</th>
<th>Gender</th>
<th>CSF Gram stain</th>
<th>Positive cultures</th>
<th>Antibiotic susceptibility a</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 a</td>
<td>Not reported</td>
<td>Bronchitis on chronic steroids, benign hyperplastic polyp on colonoscopy</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 a</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 a</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 a, 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, clindamycin, erythromycin, levofloxacin, linezolid, vancomycin</td>
<td>10</td>
<td>Radiation proctitis</td>
<td>Survived</td>
</tr>
</tbody>
</table>

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

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