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Streptococcus gallolyticus Subspecies *pasteurianus* (Biotype II/2), a Newly Reported Cause of Adult Meningitis[▽]

Amy S. Sturt,[1,2]* Liying Yang,[2] Kuldip Sandhu,[3] Zhiheng Pei,[2,3] Nicholas Cassai,[3] and Martin J. Blaser[2,3]

Stanford University, Stanford, California[1]; New York University, New York, New York[2]; and New York Veterans Affairs Healthcare System, New York, New York[3]

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We report the first case of adult meningitis confirmed to be due to *Streptococcus gallolyticus* subsp. *pasteurianus*. Phenotypically reported as *Streptococcus bovis* biotype II/2, 16S rRNA sequencing revealed *S. gallolyticus* subsp. *pasteurianus*. Because of taxonomic uncertainties, *S. gallolyticus* subsp. *pasteurianus* may be an underrecognized 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 gallolyticus*) 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. gallolyticus* 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. gallolyticus* 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/mm^3 (with 65% neutrophils, 15% bands, and 10% lymphocytes), and his glucose was 160 mg/dl. The patient was given 1 g ceftriaxone, and 2 hours later lumbar puncture showed clear, colorless cerebrospinal fluid (CSF), with a WBC of 112/mm^3 (62% neutrophils), glucose of 38 mg/dl, and protein of 282 mg/dl; no organisms were seen on Gram stain. HIV testing and three stool specimens for ova and parasites were negative.

The patient was treated for bacterial meningitis with ampicillin, vancomycin, ceftriaxone, and dexamethasone (0.15 mg/kg of body weight). A group D nonenterococcal streptococcus was identified from blood and CSF cultures. The API Rapid Strep kit (bioMérieux, Marcy l'Etoile, France) identified the organism as *S. bovis* biotype II/2, and RapID Strep (Remel, Lenexa, KS) identified it as *S. bovis* variant group D (also known as biotype II). As the cultures were sensitive to 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 1510R. From isolates 2274 (blood) 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

* Corresponding author. Mailing address: 300 Pasteur Drive, Room S-101, Stanford University, Stanford, CA 94305. Phone: (650) 725-8338. Fax: (650) 498-7011. E-mail: amysturt@standford.edu.
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TABLE 1. Phenotypic characteristics of *S. bovis* biotype II/2 (*S. gallolyticus* subsp. *pasteurianus*)

Test	Result for the study patient		% of *S. gallolyticus* subsp. *pasteurianus* strains with trait[a]
	API 20 Strep	RapID STR	
Hydrolysis of:			
Arginine	−	−	0
Esculin	+	+	100
Gallate (tannase activity)	NR[b]	NR	0
Production of:			
Acetoin	+	NR	100
β-Glucosidase	NR	NR	100
β-Glucuronidase	+	NR	100
α-Galactosidase	+	+	71
β-Galactosidase (β-Gal)	+	NR	95
β-Mannosidase	NR	NR	100
Pyrrolidonyl arylamidase	−	NR	0
Acidification of:			
Glycogen	−	NR	0
Inulin	−	−	0
Lactose	+	NR	100
Mannitol	−	−	0
Mellibiose	NR	NR	10
Raffinose	+	+	57
Starch	−	NR	14
Trehalose	+	NR	100

[a] 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, Peabody, 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 *S. pasteurianus*. The two 9324 clones differed at positions corresponding to 322, 853, and 1106 in *Escherichia coli* K-12 16S rRNA genes, likely representing true intragenomic heterogeneity (4). *Streptococcus* species usually contain four to seven rRNA operons with ≤0.2% intragenomic variation between the16S rRNA copies (4), as illustrated here. The sequencing data indicated the isolate represents *S. pasteurianus*, as our 16S rRNA genes are identical to the *S. pasteurianus* type strain and identical 16S rRNA genes have not been reported in different species. Microbiologic data also suggested the organism conforms to the phenotype previously described (Table 1) and confirmed that the strain could have been identified without 16S rRNA sequencing (22). In this study, the PCR product was cloned to provide certainty. However, sequencing of the PCR product should be sufficient for routine clinical purposes.

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. gallolyticus* 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. gallolyticus* species includes three subspecies: *S. gallolyticus* subsp. *gallolyticus*, *S. gallolyticus* subsp. *pasteurianus*, and *S. gallolyticus* subsp. *macedonicus* (22). These studies suggest *S. gallolyticus* 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, bacteremia, peritonitis, and chorioamnionitis in adults (1, 2, 10, 23). Thus far, however, there is not enough information to implicate a relationship of adult *S. gallolyticus* subsp. *pasteurianus* infection with endocarditis or colonic carcinoma. A recent report associated 63% of 11 bacteremic events with hepatobiliary 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. gallolyticus* 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. gallolyticus* 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. gallolyticus* subsp. *pasteurianus* to be confirmed by rRNA sequencing. Our patient's portal of entry may be related to radiation proctitis. The organism's capsule may explain its central nervous system tro-

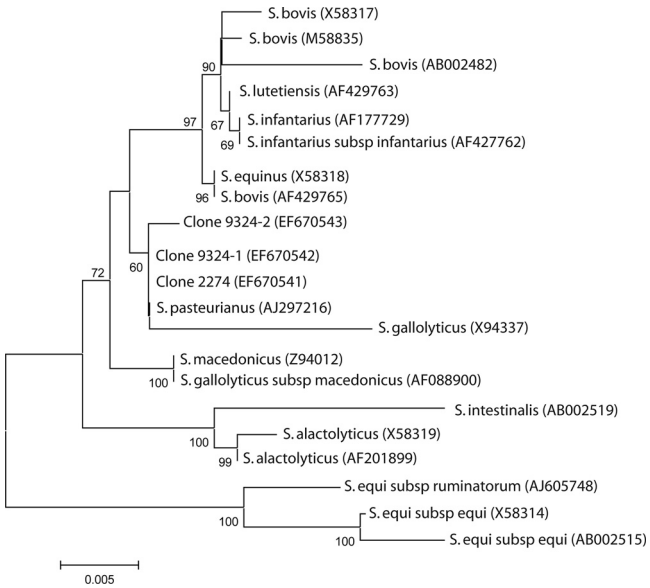

FIG. 1. Identification of clinical isolates by 16S rRNA-based phylogenetic analysis in relation to type strains of the *Streptococcus bovis* group (GenBank accession numbers are shown in parentheses). Sequences were aligned by using Greengenes, and the phylogram of the aligned sequences was generated using MEGA 3.1 with neighbor-joining methods. Bootstrap values (based on 500 replicates) are represented at each node when values are >50%, and the branch length index is represented below the phylogram.

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

Yr of report (reference)	Patient age	Gender	CSF Gram stain	Positive cultures	Antibiotic susceptibility[a]	Length of antibiotic therapy (days)	Additional clinical information	Outcome
1993 (10)	61 yrs	Male	Negative	Blood, CSF	Penicillin, cefotaxime*	Not reported	Bronchitis on chronic steroids, benign hyperplastic polyp on colonoscopy	Survived
2000 (3)	4 wks	Male	Positive	Blood, CSF	Penicillin*	18	Premature delivery	Survived
2003 (12)	3 days	Male	Positive	Blood, CSF	Penicillin*	14	Not applicable	Survived
2009 (15)	5 days	Female	Not reported	Blood, CSF	Penicillin, cefotaxime,* imipenem	14	Not applicable	Survived
Present study	75 yrs	Male	Negative	Blood, CSF	Penicillin, ceftriaxone,* clindamycin, erythromycin, levofloxacin, linezolid, vancomycin	10	Radiation proctitis	Survived

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

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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