

Reevaluation of *Streptococcus bovis* Endocarditis Cases from 1975 to 1985 by 16S Ribosomal DNA Sequence Analysis

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Studies that detected an association between *Streptococcus bovis* endocarditis and colon carcinoma have not taken into account the recently identified genetic diversity among organisms historically classified as *S. bovis*. With near full-length 16S ribosomal DNA sequence analysis, organisms cultured from the blood of endocarditis patients at the Mayo Clinic from 1975 to 1985 and previously identified as *S. bovis* or streptococcus group D nonenterococci were shown to represent *S. bovis* biotypes I (11 isolates) and II/2 (1 isolate), *S. salivarius* (1 isolate), and *S. macedonicus* (1 isolate). Two of the *S. bovis* biotype I cases were associated with colon cancer. Whether *S. bovis* biotype II or other organisms closely related to and historically identified as *S. bovis* (e.g., *S. macedonicus*) are associated with malignant (or premalignant) colon lesions in humans remains to be definitively determined.

Streptococcus bovis is the causative agent of 5 to 14% of cases of endocarditis (4). An association between fecal carriage of *S. bovis* and carcinoma of the colon has been recognized for 2.5 decades (11), and an association between *S. bovis* endocarditis and carcinoma of the colon has been appreciated for a similar time period (1, 9). *S. bovis* NCTC 8133 and ATCC 41344 have been demonstrated to adhere to intestinal epithelial cells and stimulate the production of cytokines that promote vasodilation and increased capillary permeability, creating a potential portal of entry for microbes (5). *S. bovis* NCTC 8133 has been further shown to be a promoter of early preneoplastic lesions in the colons of rats (6). Importantly, since the early 1980s, genetic and biochemical diversity has been noted among organisms classically designated *S. bovis*; it is now understood that organisms historically categorized as *S. bovis* (7) may represent one of several biotypes of *S. bovis* or even non-*S. bovis* streptococci. Because of the relationship between *S. bovis* and carcinoma of the colon, as well as that between *S. bovis* and endocarditis, it may be important to accurately identify these organisms.

Recently published studies either have not addressed the diversity of *S. bovis* isolates in the context of *S. bovis* endocarditis (4, 12, 14) or have addressed this issue in a preliminary and often contradictory fashion (2, 3). Prior to the description of *S. gallolyticus*, Ruoff et al. reported that among *S. bovis* biotypes identified by the API Rapid Strep system (Analytab Products, Plainview, N.Y.) and cellular fatty acid content, biotype I was more likely than biotype II to be associated with both endocarditis and malignant or premalignant colonic lesions (15). Following the description of *S. gallolyticus*, Devriese et al. used whole-cell protein analysis to show that all six

bacterial isolates studied, which were derived from patients with endocarditis and identified by conventional techniques as *S. bovis*, were in fact *S. gallolyticus* (3). Devriese et al. suggested that *S. gallolyticus* is more likely to be involved in human infections than is *S. bovis* (3). Schlegel et al. further suggested that most of the mannitol-positive group D streptococci isolated from blood, which had been reported to be responsible for human endocarditis associated with colonic cancer (15), might actually be *S. gallolyticus* (16). More recently, however, Clarridge et al. used partial 16S ribosomal DNA (rDNA) sequence analysis to show that none of five bacterial isolates associated with human endocarditis and identified by conventional techniques as *S. bovis* were *S. gallolyticus* (2). In their study, endocarditis isolates were all mannitol negative and were *S. bovis* biotype I (1 isolate), *S. bovis* biotype II/1 (1 isolate), or *S. bovis* biotype II/2 (3 isolates) (2).

The purpose of this study was to determine whether organisms cultured from the blood of endocarditis patients at the Mayo Clinic from 1975 to 1985 and previously identified as *S. bovis* represent *S. bovis* biotype I, *S. bovis* biotype II/1, *S. bovis* biotype II/2, *S. gallolyticus*, *S. salivarius*, or other recently described streptococci, as determined by near full-length 16S rDNA sequence analysis.

The records of the Mayo Clinic endocarditis database (1975 to 1985) were reviewed to identify *S. bovis* or streptococcus group D nonenterococcus endocarditis cases. We identified 14 cases (patient ages, 45 to 81; 9 males; 12 had native-valve involvement, and 2 had prosthetic-valve involvement) for which the infecting microorganism had been archived at -70°C (Table 1).

16S rDNA PCR amplification and bidirectional sequencing of 1,430 nucleotides were performed using previously described cycling conditions and primers (10) and previously described PCR mixtures (13). Sequence data were analyzed using Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, Mich.).

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TABLE 1. Characteristics of the 14 patients with endocarditis caused by bacteria previously characterized as *S. bovis* or streptococcus group D nonenterococci^a

Patient	Age	Gender	Date of diagnosis	Valve involved	N or P valve	Gastrointestinal pathology	Organism identification (initial)	Organism identification by 16S rDNA sequence analysis
1	50	M	09/04/75	A/MI	N	Not assessed	SDNE	<i>S. bovis</i> biotype I
2	57	F	12/28/75	A/MI	N	Not assessed	SDNE	<i>S. bovis</i> biotype I
3	70	M	07/27/76	A	N	Not assessed ^b	SDNE	<i>S. bovis</i> biotype II/2
4	60	M	11/01/76	A	N	Gastric adenomatous polyps	SDNE	<i>S. bovis</i> biotype I
5	59	F	02/08/77	A/MI	N	Normal colon X ray, primary biliary cirrhosis	SDNE	<i>S. bovis</i> biotype I
6	45	M	08/30/77	A	N	Ulcerative colitis	SDNE	<i>S. bovis</i> biotype I
7	62	M	11/07/77	MI	N	Sigmoid colon adenocarcinoma	SDNE	<i>S. bovis</i> biotype I
8	78	F	12/01/77	A	N	Normal colonoscopy	SDNE	<i>S. bovis</i> biotype I
9	70	M	06/29/78	MI	N	Cecal polyp	SDNE	<i>S. macedonicus</i>
10	58	F	01/03/80	A	N	Ulcerative colitis	<i>S. bovis</i>	<i>S. bovis</i> biotype I
11	74	M	07/08/80	MI/T	N	Sigmoid colon villous adenoma	<i>S. bovis</i>	<i>S. bovis</i> biotype I
12	72	F	12/06/80	A	P	Not assessed	<i>S. bovis</i>	<i>S. salivarius</i>
13	81	M	09/09/83	A	P	Sigmoid colon diverticulosis	<i>S. bovis</i>	<i>S. bovis</i> biotype I
14	73	M	02/10/85	A/Pul	N	Ascending colon adenocarcinoma	<i>S. bovis</i>	<i>S. bovis</i> biotype I

^a M, male; F, female; N, native valve; P, prosthetic valve; MI, mitral valve; T, tricuspid valve; A, aortic valve; Pul, pulmonary valve; SDNE, streptococcus group D nonenterococcus.

^b This patient had iron deficiency anemia and occult blood in the stool.

The 16S rDNA sequences of 11 isolates were identical to ($n = 10$) or different in one base pair from ($n = 1$) that of the type strain *S. bovis* ATCC 43143 (Fig. 1). These 11 isolates belonged to cluster 2a, which is *S. bovis* biotype I as defined by Clarridge et al. (2). One isolate (isolate 12) was most closely related to *S. salivarius*, and another isolate (isolate 9) was most closely related to *S. macedonicus* (17). The sequence of one isolate (isolate 3) was identical to the available sequence of VAMC blood3395. This places the isolate in cluster 2b, which is *S. bovis* biotype II/2 as defined by Clarridge et al. (2). None of the isolates studied clustered with *S. bovis* biotype II/1 as defined by Clarridge et al. (2), and none clustered with *S. gallolyticus*. All isolates produced acid from trehalose; all except two (isolates 3 and 12) produced acid from mannitol; all except one (isolate 12) were urease negative and bile esculin positive.

Of the 14 patients studied, 2 had colon cancer; 1 (case 7) of these was diagnosed simultaneously with endocarditis, and the other (case 14) was diagnosed 1 month prior to the onset of endocarditis symptoms. Six patients had other gastrointestinal pathologies (ulcerative colitis [cases 6 and 10], sigmoid colon villous adenoma [case 11], a cecal polyp [case 9], gastric adenomatous polyps [case 4], and sigmoid colon diverticulosis [case 13]). Two further patients had negative colonoscopic or double-contrast barium enema studies; of these patients, one had primary biliary cirrhosis (case 5). Four patients (cases 1, 2, 3, and 12) did not undergo colonic evaluation; however, one of these four (case 3) had findings indicative of a gastrointestinal-tract abnormality (iron deficiency anemia and occult blood detected in the stool) at the time of endocarditis diagnosis.

This is the largest series of *S. bovis* endocarditis isolates subjected to near full-length 16S rDNA sequence analysis. The organisms involved in these endocarditis cases were shown to be predominantly *S. bovis* biotype I (11 isolates), followed by *S. bovis* biotype II/2 (1 isolate), *S. salivarius* (1 isolate), and *S. macedonicus* (1 isolate). Our findings support earlier findings based on the API Rapid Strep system and cellular fatty acid studies that *S. bovis* biotype I is most frequently associated with endocarditis in humans (15) and refute the suggestion of

Devriese et al. that *S. gallolyticus* is a more common pathogen than *S. bovis* in human endocarditis (3).

It has previously been observed that *S. salivarius* can be misidentified as *S. bovis* (15). Since *S. salivarius* has been shown to be less likely to be associated with colonic lesions or endocarditis than *S. bovis* (15), differentiating *S. salivarius* from *S. bovis*, which can be accomplished by the methods described herein, can provide clinically useful information. Our *S. salivarius* endocarditis patient did not have known colonic pathology.

This is the first description of human infection caused by *S. macedonicus*, an organism originally isolated from naturally fermented Greek Kasser cheese (8, 17). It is unknown whether *S. macedonicus* endocarditis is associated with colonic carcinoma; our single case was associated with a cecal polyp.

16S rDNA sequencing and sequence analysis are being increasingly used in clinical microbiology laboratories for bacterial identification. This tool enables accurate species and subspecies level identification of *S. bovis* and closely related bacteria such as *S. salivarius*, *S. macedonicus*, and *S. gallolyticus*. The association or lack thereof of endocarditis with colon pathology needs to be considered when patients with endocarditis caused by these organisms are evaluated. The isolate determined to be a promoter of early preneoplastic lesions in the colons of rats is biotype II/1 (Marie Scholler-Guinard, personal communication) (6). Whether other biotypes of *S. bovis* and/or other organisms closely related to and historically identified as *S. bovis* (e.g., *S. macedonicus*) would yield similar results in this rat model remains to be determined. The isolates determined to adhere to intestinal epithelial cells and stimulate the production of cytokines that promote vasodilation and increased capillary permeability (5) include the aforementioned biotype II/1 isolate, as well as ATCC 31344, which, on the basis of 16S rDNA sequence analysis (GenBank accession number AF104115), was determined to be biotype II/2 as defined by Clarridge et al. (2). Based on our results for humans, *S. bovis* biotype I is associated with both endocarditis and malignant and premalignant colon lesions. Whether *S. bovis*

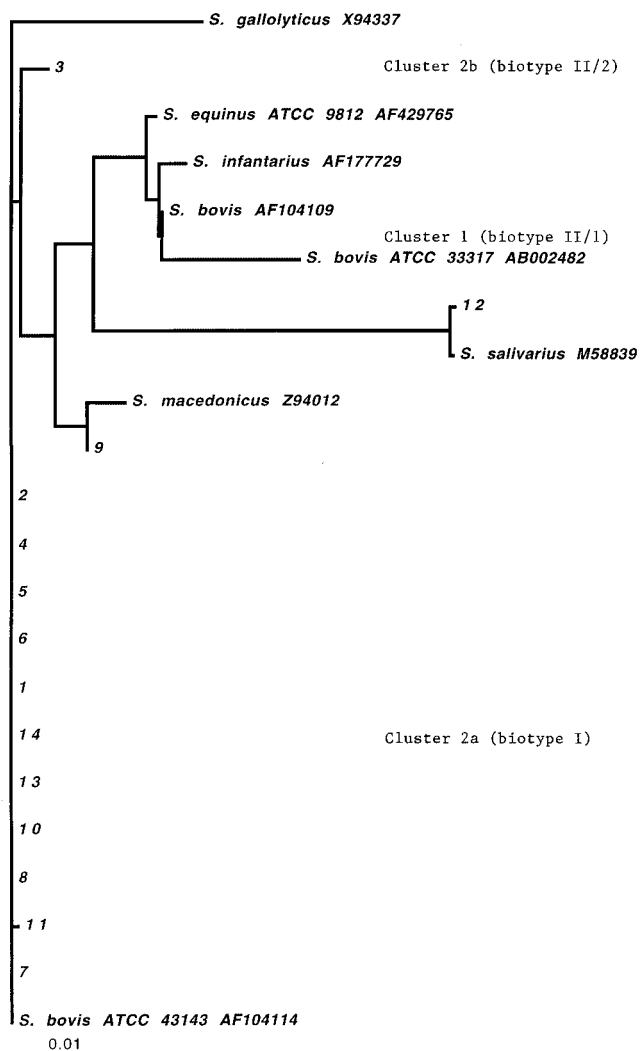


FIG. 1. Dendrogram of phylogenetic relationships among study isolates, American Type Culture Collection type strains, and GenBank entries. Distances were calculated by the neighbor-joining method. The bar indicates a 1% difference measured horizontally. Isolate numbers correspond to case numbers cited in the text. The sequence of isolate 12 had one base different from that of *S. salivarius* M58839. Isolate 9 had five bases different from *S. macedonicus* ATCC 43143. Clusters are indicated as defined by Clarridge et al. (2).

biotype II or other organisms closely related to and historically identified as *S. bovis* (e.g., *S. macedonicus*) are associated with malignant and premalignant colon lesions in humans remains to be definitively determined.

Nucleotide sequence accession numbers. 16S rDNA sequences have been deposited in GenBank under accession

numbers AF459434 and AF459433 for isolates 9 and 12, respectively.

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