

Bacteremia with *Streptococcus bovis* and *Streptococcus salivarius*: Clinical Correlates of More Accurate Identification of Isolates

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Two biotypes of *Streptococcus bovis* can be identified by laboratory testing and can be distinguished from the phenotypically similar organism *Streptococcus salivarius*. We assessed the clinical relevance of careful identification of these organisms in 68 patients with streptococcal bacteremia caused by these similar species. *S. bovis* was more likely to be clinically significant when isolated from blood (89%) than was *S. salivarius* (23%). There was a striking association between *S. bovis* I bacteremia and underlying endocarditis (94%) compared with that of *S. bovis* II bacteremia (18%). Bacteremia with *S. bovis* I was also highly correlated with an underlying colonic neoplasm (71% of patients overall, 100% of those with thorough colonic examinations) compared with bacteremia due to *S. bovis* II or *S. salivarius* (17% overall, 25% of patients with thorough colonic examinations). We conclude that careful identification of streptococcal bacteremic isolates as *S. bovis* biotype I provides clinically important information and should be more widely applied.

The association between bacteremia caused by *Streptococcus bovis* and gastrointestinal disease, particularly colonic malignancy, is well documented (8-10, 12, 14). However, most previous studies have not reported the physiological characteristics of *S. bovis* strains in detail, and thus the distribution of isolates among the different biotypes of *S. bovis* is unknown. We have previously observed similarities in the physiological reactions of *S. bovis* and the viridans group species *Streptococcus salivarius* which could result in the erroneous identification of *S. salivarius* as *S. bovis* (16) and could confound the relationship between bacteremia caused by *S. bovis* and specific disease states.

In a study of phenotypic characteristics and genetic relationships of *S. bovis* and *S. salivarius*, Coykendall and Gustafson (3) noted that in spite of phenotypic similarities, *S. salivarius* and *S. bovis* strains were genetically distinct at the species level. Previous studies of physiological characteristics of *S. bovis* isolates have identified two biotypes. *S. bovis*, also called *S. bovis* I, was differentiated from *S. bovis* variant, also referred to as *S. bovis* II, on the basis of mannitol fermentation and starch hydrolysis reactions and the ability to produce polysaccharide from sucrose (5, 13). In the commercially available identification system used by Coykendall and Gustafson, *S. bovis* variant (*S. bovis* II) is further separated into two subbiotypes, *S. bovis* II/1 and II/2. Coykendall and Gustafson found that *S. bovis* and one of the *S. bovis* variant subbiotypes constituted a genetically defined species while strains of the other *S. bovis* variant subbiotype appeared to form a subspecies of *S. bovis* (3). Knight and Shlaes (11) found that all five *S. bovis* variant isolates they studied could be included in the same DNA homology group with *S. bovis* isolates from humans. Since these authors did not use the same methods for biotyping as Coykendall and Gustafson, it is not known which subbiotypes their *S. bovis* variant strains resembled.

Thus *S. salivarius*, *S. bovis*, and *S. bovis* variant strains are organisms with phenotypic similarities, but they may be

differentiated by extended physiological characterization. Since many previous studies have used only a minimal number of characteristics to identify isolates as *S. bovis*, no data exist on possible differences in the association of gastrointestinal cancer with the different biotypes of *S. bovis* or the physiologically similar *S. salivarius*. We therefore collected and identified 71 blood culture isolates of *S. bovis*, *S. bovis* variant, and *S. salivarius* from patients at the Massachusetts General Hospital. A review of the charts of the patients was then carried out to assess the clinical relevance of a more careful identification of these similar streptococcal isolates.

MATERIALS AND METHODS

Bacteriological studies. Most of the strains studied were consecutive isolates, collected from blood cultures in the Massachusetts General Hospital Bacteriology Laboratory during the period April 1982 through March 1987; 15 strains were isolated from blood cultures received in the laboratory prior to this period. Isolates were stored frozen in horse blood at -70°C and after thawing were routinely propagated on brucella agar containing 5% horse blood (GIBCO Diagnostics, Madison, Wis.) at 35°C in an atmosphere containing 5% CO_2 .

The physiological and serological characteristics and cellular fatty acid content of 15 strains included in this study have been reported previously (16); these techniques were applied to an additional 34 isolates in the present report. All 71 strains were identified with the API Rapid Strep system (Analytab Products, Plainview, N.Y.).

Patient chart review. Over the period of the study, 40 patients hospitalized at Massachusetts General Hospital had bacteremia caused by *S. bovis* and 31 patients had bacteremia caused by *S. salivarius*. Medical records were available for review from all but three of these patients, one with bacteremia due to *S. salivarius* and two with bacteremia due to *S. bovis* variant. The medical records were reviewed by using a standardized protocol to determine the demographic characteristics of the patients, in-hospital mortality, and the

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TABLE 1. Demographic characteristics of patients with *S. bovis* or *S. salivarius* isolated from blood cultures

Isolate	No. of patients ^a		Age (yr)		
	Male	Female	Mean	Median	Range
<i>S. bovis</i>					
All biotypes	21	17	66.9	72	1-85
Biotype I	10	7	69.2	71	50-85
Biotype II (all subbiotypes)	11	10	65.1	72	1-85
Biotype II/1	6	6	58.4	70.5	1-85
Biotype II/2	5	4	74.0	77	62-83
<i>S. salivarius</i>	22	8	40.8	49.5	0-87

^a One isolate from each patient.

clinical significance of the blood isolates, by using the criteria of Broome et al. (1). Significant isolates were defined as those occurring in two or more separate blood cultures associated with clinical evidence of endocarditis or septicemia or those occurring in a single blood culture from a patient with a clinically obvious infection. Blood isolates were regarded as insignificant if they occurred in a single culture from a patient without clinical evidence of infection or if they were recovered in association with other bacterial species usually present on skin or in the oral cavity. Endocarditis was diagnosed as previously defined (2), if streptococci were recovered from two or more blood cultures and there was either a compatible clinical syndrome (a new or changing cardiac murmur, evidence of peripheral embolism, or cutaneous manifestations of endocarditis) or histopathologic confirmation of endocarditis at surgical or postmortem examination. Colonic lesions were defined as associated with the bacteremia if they were diagnosed during the acute hospitalization or during a 6-month follow-up period. In addition to colonic adenocarcinoma, the presence of adenomatous polyps or villous adenomas was recorded because these lesions are considered to be premalignant (17). A possible hepatobiliary origin of bacteremia was recorded if the patient had clinically evident cholecystitis or cholangitis or pathologically documented cirrhosis with portasystemic shunting at the time of bacteremia.

Statistical analyses. Two-way tables comparing the frequencies of categorical events were analyzed by the chi-square statistic or the Fisher exact test. A two-sided probability of <0.05 was considered statistically significant.

RESULTS

Bacteriological characterization of isolates. The distinguishing features of *S. bovis*, *S. bovis* variant, and *S. salivarius*

have been detailed previously (16) and are briefly summarized here. *S. bovis* (13 strains) and *S. bovis* variant (17 strains) all gave positive reactions in tests with conventional tubed media for the determination of esculin hydrolysis in the presence of 40% bile and acidification of sucrose, lactose, and raffinose. These isolates failed to grow in Todd-Hewitt broth containing 6.5% NaCl, did not produce acid from sorbitol, arabinose, or sorbose, and were unable to hydrolyze arginine. The majority of *S. bovis* strains produced acid from mannitol and inulin, hydrolyzed starch, and formed polysaccharide when grown on a sucrose-containing medium; *S. bovis* variant isolates were devoid of these characteristics.

The 19 *S. salivarius* strains examined by tests with conventional tubed media all acidified lactose and failed to grow in Todd-Hewitt broth with 6.5% NaCl. They were inactive on sorbitol, arabinose, sorbose, mannitol, and arginine. The majority of strains fermented sucrose, raffinose, and inulin and produced polysaccharide on a sucrose-containing medium. Three isolates gave a positive bile esculin reaction, and one isolate hydrolyzed starch.

After observing excellent agreement between API Rapid Strep identifications and those generated by conventional tests of the 49 isolates described above, we relied solely on the Rapid Strep system for identification of the remaining strains. In only a few cases was the Rapid Strep identification inconclusive, involving a choice between *S. bovis* variant and *S. salivarius*. For these strains, the presence (characteristic of *S. salivarius*) or absence of eicosenoic acid was determined by gas-liquid chromatography as described previously (16).

The Rapid Strep system distinguishes two subbiotypes of *S. bovis* variant, referred to as *S. bovis* II/1 and II/2. The tests used for this differentiation (acidification of starch and glycogen and other enzymatic activities) are not usually included among conventional differential tests for streptococci and, therefore, distinction of subbiotypes of *S. bovis* variant was not accomplished with tubed media.

Analysis of patient data. The demographic characteristics of the study population are shown in Table 1. Of 38 isolates of *S. bovis* causing bacteremia, 17 were biotype I, 12 were subbiotype II/1, and 9 were subbiotype II/2. The sex and age distributions of patients infected with the *S. bovis* biotypes were not significantly different, except that two infants had bacteremia with *S. bovis* subbiotype II/1 related to line sepsis. The patients with bacteremia caused by *S. salivarius* were significantly younger than those with bacteremia caused by *S. bovis*.

Several clinical characteristics of the study population are detailed in Table 2. Isolation of *S. bovis* from a blood culture

TABLE 2. Clinical characteristics of patients with *S. bovis* or *S. salivarius* isolated from blood cultures

Isolate	No. of isolates		No. of patients with:			No. of patients who died during hospitalization
	Total	Clinically significant	Endocarditis	Malignant or premalignant colonic lesion	Hepatobiliary origin of bacteremia	
<i>S. bovis</i>						
All biotypes	38	34	19	15	9	9
Biotype I	17	17	16	12	0	3
Biotype II (all subbiotypes)	21	17	3	3	9	6
Biotype II/1	12	10	1	1	7	4
Biotype II/2	9	7	2	2	2	2
<i>S. salivarius</i>	30	7	3	1	4	3

was more likely to reflect a clinically significant infection (89%) than was isolation of *S. salivarius* (23%, $P < 0.001$); this was true for each of the biotypes of *S. bovis* as well. Of 17 patients with bacteremia caused by *S. bovis* I, 16 (94%) had bacterial endocarditis, in contrast to 3 of 17 patients (18%) with clinically significant bacteremia due to *S. bovis* II ($P < 0.001$) and 3 of 7 patients (43%) with bacteremia due to *S. salivarius* ($P < 0.03$). There were no significant differences in hospital mortality related to the species of streptococci isolated.

Of 17 patients with bacteremia due to *S. bovis* I, 12 (71%) had associated malignant or premalignant colonic lesions. This association was significantly higher than that for patients with bacteremia caused by *S. bovis* II or *S. salivarius* (4 of 24 patients, 17%; $P < 0.01$). The more frequent association of colonic neoplasms with bacteremia caused by *S. bovis* I than with bacteremia caused by the other isolates was not explained by more thorough colon examinations. The large intestines were examined by colonoscopy, exploratory laparotomy, or autopsy in 12 patients with bacteremia caused by *S. bovis* I, and malignant or premalignant lesions were identified in all 12 (100%). In contrast, only 4 of 16 patients (25%) with bacteremia caused by the other organisms who underwent similar examination had colonic neoplasms ($P < 0.001$).

Clinically significant bacteremia caused by *S. bovis* II/1 or *S. salivarius* was more likely to have a hepatobiliary origin (11 of 17 patients, 65%) than was bacteremia caused by *S. bovis* I (none of 17 patients, $P < 0.001$).

DISCUSSION

In a recent review of the literature, Klein and co-workers (10) noted that 37% of a total of 251 patients with septicemia caused by *S. bovis* had evidence of colonic disease, including carcinoma, polyps, or unidentified colonic masses. Of 34 patients with prospective lower gastrointestinal tract evaluation, 50% were found to exhibit these conditions. These observations are similar to those of our study in that 39% of 41 patients with clinically significant bacteremia caused by *S. bovis* or *S. salivarius* had colonic neoplasms and 57% of 28 patients who underwent thorough colonic examination had such lesions. However, more detailed identification of streptococcal blood isolates to the biotype level revealed an even more striking association between bacteremia caused by *S. bovis* I and colonic neoplasia. Seventy-one percent of patients with bacteremia due to *S. bovis* I overall and 100% (12 of 12) of such patients who underwent thorough colonic examination had neoplasms detected. In contrast, only 25% of the remaining 16 patients with bacteremia caused by other biotypes of *S. bovis* or by *S. salivarius* had neoplasms detected on thorough colonic examination. These data suggest that identification of *S. bovis* biotype I strains among streptococcal isolates causing bacteremia identified patients at even higher risk of having colonic neoplasia than previously documented and that all patients with bacteremia caused by this organism should undergo colonoscopy unless contraindicated.

The striking association between bacteremia caused by *S. bovis* biotype I and both colonic neoplasia (71%) and bacterial endocarditis (94%), compared with bacteremias caused by the closely related organisms *S. bovis* variant and *S. salivarius*, suggests the possibility of specific bacterium-host cell interactions involving *S. bovis* biotype I organisms. Such a specific interaction could involve, for example, selective adherence of this biotype to surface receptors on

neoplastic colonic cells or cardiac endothelium. Further studies will be needed to address the possibility of such specific interactions more directly.

Bacteremia due to *S. salivarius* was less likely to reflect a clinically significant infection than was bacteremia due to *S. bovis*. Nevertheless, of seven patients with clinically significant bacteremia caused by *S. salivarius*, three patients had bacterial endocarditis and one had an associated colonic neoplasm. Roses et al. (15) reported a case of endocarditis due to *S. salivarius* associated with colonic adenocarcinoma, and Hoecker and co-workers (7) reported six cases of sepsis due to *S. salivarius* in children with underlying malignant diseases. Our data suggest a possible hepatobiliary origin for many clinically significant infections with *S. salivarius* and *S. bovis* subbiotype II/1, an anatomical correlation not previously recognized for either of these organisms.

We conclude that accurate differentiation between isolates of *S. bovis* and *S. salivarius* and biotyping of isolates of *S. bovis* provide clinically useful information. Problems in identifying these physiologically similar organisms with presumptive tests have been discussed previously (16), but our experiences and those of others (6) have demonstrated the utility of the API Rapid Strep system for identification of these organisms; other commercially available systems may also be of use for this purpose (4). No clear distinction in the clinical significance of the subbiotypes of *S. bovis* II could be observed from our data. Our findings suggest that identification of *S. bovis* to the level of biotype I or II and distinction between these organisms and *S. salivarius* should be more widely applied.

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