

An Update on the *Streptococcus bovis* Group: Classification, Identification, and Disease Associations

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The *Streptococcus bovis* group has undergone significant taxonomic changes over the past 2 decades with the advent of new identification methods with higher discriminatory power. Although the current classification system is not yet embraced by all researchers in the field and debate remains over the performance of molecular techniques for identification to the species level within the group, important disease associations for several members of the group have been clarified. Here, we provide a brief overview of the history of the *S. bovis* group, an outline of the currently accepted classification scheme, a review of associated clinical syndromes, and a summary of the performance and diagnostic accuracy of currently available identification methods.

The history of the *Streptococcus bovis* group is complicated and confusing due to conflicting classical distinctions based on imperfectly differentiating phenotypic attributes and due to modern disagreements concerning the optimal molecular methods for identification to the species level. Consequently, even the current classification system remains subject to debate and is not accepted by all researchers in the field. In this Minireview, we provide a brief overview of the history of the *S. bovis* group and description of a current classification system, followed by a discussion of disease associations. We conclude with an overview of currently available diagnostic methods for species- and subspecies-level identification.

A BRIEF TAXONOMIC HISTORY OF THE *S. BOVIS* GROUP

In her seminal work, Rebecca Lancefield defined group D carbohydrate antigen reactivity among a collection of beta-hemolytic streptococci isolated from cheese (1). Studies of a more diverse group of streptococci over subsequent years revealed that a number of nonhemolytic and alpha-hemolytic isolates from both animal and human sources also demonstrated group D antigen reactivity and shared important characteristics with one another (2). Within this serologic group were bovine and dairy-associated streptococci, some of which were referred to as *Streptococcus bovis* or *S. bovis* group, based loosely on shared characteristics with the *S. bovis* strain that was originally defined by Orla-Jensen in 1919 (3, 4). *Streptococcus equinus*, *Streptococcus faecalis* and *Streptococcus faecium* strains were also within the D serologic group, although the latter two were separable from the other group D streptococci based on their ability to grow in 6.5% NaCl, hydrolyze arginine, and decarboxylate tyrosine, and they were ultimately renamed enterococci (3, 5). In the early 1960s, classification systems were developed that placed *S. bovis* into a more defined scheme among the group D streptococci (3).

Though the founding members of the group D streptococci were animal in origin, there was an emerging appreciation that human-origin group D isolates were likely important agents of human infection. Facklam emphasized that accurate methods of identification to the species level were essential to understanding the species distribution of the group D streptococci among human infections and their antimicrobial susceptibilities (6). Potentially important disease associations, along with better methods for identification to the species level, stimulated much interest in

the group D streptococci, which led to the current classification system.

CURRENT TAXONOMY OF THE *S. BOVIS* GROUP

Following the taxonomic separation of the enterococci from the group D streptococci, a set of strains referred to as the *S. bovis*-*S. equinus* complex remained (7). In older and in some current literature, strains of this complex have been classified on the basis of their ability to ferment mannitol. Those strains that could ferment mannitol were designated biotype I, and those that could not, as biotype II (also referred to as *S. bovis* variant in some literature). Biotype II was further divided into biochemical subtypes II/1 and II/2 based initially on bile-esculin reaction, acidification of trehalose, and hydrolysis of starch and subsequently on sequence-based analysis (8–11). These biotype classifications retain relevance due to their frequent citation in the literature.

A current taxonomic system that follows recent developments in the literature supplemented by genetic analysis includes seven divisions within the group D streptococci (biotype classifications are shown in parentheses but are not part of official taxonomic nomenclature): *S. equinus*, *Streptococcus infantarius* subsp. *coli* (biotype II/1), *S. infantarius* subsp. *infantarius* (biotype II/1), *Streptococcus alactolyticus*, *Streptococcus gallolyticus* subsp. *gallolyticus* (biotype I), *S. gallolyticus* subsp. *pasteurianus* (biotype II/2), and *S. gallolyticus* subsp. *macedonicus* (7, 12, 13). Though this nomenclature is still subject to debate and has not yet been universally embraced, we will use this system for consistency with other recent attempts to harmonize analysis of the literature (14).

DISEASE ASSOCIATIONS OF THE *S. BOVIS* GROUP

It had been appreciated since at least the early 1950s that group D streptococci were a cause of infective endocarditis. However, the detailed species associations were not well understood, and some

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early nonenterococcal *S. bovis* isolates may have been classified either as enterococci due to their bile insolubility and ability to hydrolyze bile esculin or as *Streptococcus viridans* due to their penicillin susceptibility (15). As more refined methods to separate the enterococci and nonenterococcal group D organisms were developed, it became clear that the nonenterococcal group D strains were indeed a significant cause of endocarditis.

An association between colorectal carcinoma and group D endocarditis was appreciated at least as early as 1951 (16). In 1974, Hopes and Lerner (17) reported a case series suggesting a relationship between nonenterococcal *S. bovis* and colorectal carcinoma, as well as other gastrointestinal diseases. Klein et al. (15) studied the relationship between gastrointestinal colonization with *S. bovis* and a variety of gastrointestinal conditions, including colonic carcinoma, inflammatory bowel disease, peptic-ulcer disease, diverticular disease, and gastrointestinal bleeding of unknown etiology using the methods of Facklam for identification to the species level (6). These authors found *S. bovis* in fecal cultures from 35 of 63 (56%) patients with colonic carcinoma, representing a significantly greater proportion than in any other group studied. A review of earlier literature suggested that possibly six of nine previous cases that had reported an association between colorectal carcinoma and infective endocarditis involved an *S. bovis* group organism (15).

In 1989, Ruoff and colleagues (18), using more accurate identification techniques, found striking correlations between *S. bovis* biotype I (*S. gallolyticus* subsp. *gallolyticus*) bacteremias and infective endocarditis (94%) and colonic neoplasm (71% of patients overall and 100% of those who underwent thorough examination). These rates were substantially greater than those associated with the *S. bovis* biotype II isolates in the study (18% association with endocarditis, and 17% overall association with colonic carcinoma), suggesting the possibility of a specific disease association with *S. gallolyticus* subsp. *gallolyticus*. Corredoira et al. (19) studied *S. bovis* isolates that had been collected over a 16-year period and found that 74% (31 of 42) of *S. bovis* biotype I (*S. gallolyticus* subsp. *gallolyticus*) isolates were associated with endocarditis and 57% (24/42) were associated with colonic tumors. Similar to those seen in Ruoff et al. (18), these rates were significantly greater than the rates associated with *S. bovis* biotype II isolates (19). Another study of 58 consecutive *S. bovis* bacteremia isolates found that 9 of 21 *S. gallolyticus* subsp. *gallolyticus* isolates were associated with infective endocarditis, and 2 of 21 of these isolates were associated with colonic carcinoma; however, only a limited number of patients in this study underwent colonoscopy, leading to a potential underestimation in the actual association (9).

In total, much evidence has accumulated that supports a specific association of *S. gallolyticus* subsp. *gallolyticus* with infective endocarditis and colorectal cancer. A recent thorough meta-analysis (14) found that patients who had *S. bovis* biotype I (*S. gallolyticus* subsp. *gallolyticus*) infection had a strongly increased risk of having colorectal carcinoma (odds ratio, 7.26; 95% confidence interval, 3.94 to 13.36) compared with those who had *S. bovis* biotype II infections. This meta-analysis also found that *S. bovis* biotype I was a much more common cause of infective endocarditis than *S. bovis* biotype II. The authors point out that these strong disease associations underscore the importance of proper identification to the species level and classification of *S. bovis* isolates by clinical microbiology laboratories and the interpretation by physicians.

Interestingly and importantly, a different pattern of disease association for *S. gallolyticus* subsp. *pasteurianus* is implied by recent literature. In 1997, Cohen et al. reported one case of *S. bovis* biotype II isolated from cerebrospinal fluid from a patient with meningitis and another isolated from a patient with a subdural empyema (20). The authors performed a literature review and found 14 previously reported cases of meningitis attributed to *S. bovis*. Since the time of the Cohen et al. review (20), at least 16 additional cases of adult and pediatric meningitis attributed to *S. bovis* biotype II/2 (*S. gallolyticus* subsp. *pasteurianus*) have been documented, though the actual number may be much larger, given that, in many cases, the isolates were not identified to the subspecies level (21–31). Notably, most of these cases occurred in patients who lacked an identified colonic neoplasm, and the meningitis occurred without an identified bacteremia in some cases. Interestingly, there was an association with *Strongyloides stercoralis* infection in 14 cases of *S. gallolyticus* meningitis reported by van Samkar et al., suggesting an underlying mechanism by which cerebrospinal fluid infection occurred, though it should be noted that not all isolates in this case series were of clearly defined subspecies (31). The emerging picture from this literature suggests that *S. gallolyticus* subsp. *pasteurianus* may indeed be a significant (though rare) cause of meningitis in patients without identified colonic neoplasm and that it is a pathogen that may have an underappreciated significance as an agent of systemic disease in infants.

Given a new understanding of the techniques and strategies that are required to separate members of the *S. bovis* group, it is clear that, in some cases, accurate disease associations have been obscured in older and possibly in some current literature and that a re-examination of isolates in light of current taxonomy is required where possible.

DIAGNOSTIC METHODS FOR THE IDENTIFICATION OF SPECIES AND SUBSPECIES WITHIN THE *S. BOVIS* GROUP

The specific disease associations within the *S. bovis* group described above underscore the importance of accurate species- and subspecies-level classification of clinical isolates. However, the complexity of taxonomic changes over the last decade has made it difficult to ensure accurate and complete classification in many cases. Contributing factors have included the absence of a curated sequencing database, the lack of revised nomenclature in culture collection deposits, and the irregularity (or lack) of updates to commercial phenotypic-based identification databases, in part due to the lengthy FDA regulatory approval process to which such changes are subject. Regular taxonomic review and incorporation of nomenclature changes into routine clinical microbiology reporting are now mandated by certain laboratory accrediting agencies, such as the College of American Pathologists, because of the potential effects that such changes may have on antimicrobial choice and/or interpretive criteria (CAP Accreditation Program item MIC.11375) (32). It is therefore the responsibility of the laboratory to understand currently accepted taxonomy and the limitations of contemporary diagnostic platforms in order to provide accurate organism identification that may impact clinical guidance for specific disease syndromes.

The performance of phenotypic, biochemical, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), and sequencing-based methods for species- and subspecies-level identification within the *S. bovis* group are

TABLE 1 Summary of commercial identification databases and the manufacturer's listed coverage of species and subspecies within the *S. bovis* group^a

Species or subspecies	Coverage for biochemical system			Coverage for MALDI-TOF MS system			
	API 20 strep	Rapid ID 32 strep	Vitek 2 GP ID card	Bruker Biotyper ^b (no. of representative mass spectral profiles)	Vitek MS 2.0	Vitek MS 3.0 ^c	Saramis
<i>S. alactolyticus</i> ^d	No	Yes	Yes	Yes (1)	Yes ^c	Yes	No
<i>S. equinus</i> ^d	Yes	No	Yes	Yes (2)	Yes ^c	Yes	Yes
<i>S. gallolyticus</i> subsp. <i>gallolyticus</i> ^d	Yes	No	Yes	Yes (2)	Yes ^c	Yes	Yes
<i>S. gallolyticus</i> subsp. <i>macedonicus</i> ^d	No	No	No	Yes (2)	No	No	Yes
<i>S. gallolyticus</i> subsp. <i>pasteurianus</i> ^d	Yes	No	Yes	Yes (3)	Yes ^c	Yes	Yes
<i>S. infantarius</i> subsp. <i>coli</i> ^d	Yes	No	Yes	Yes (6) ^e	Yes ^f	Yes	Yes
<i>S. infantarius</i> subsp. <i>infantarius</i> ^d	Yes	No	Yes	Yes (1) ^g	Yes ^f	Yes	Yes
<i>S. bovis</i>	No	Yes	No	No	No	No	No
<i>S. gallolyticus</i>	No	No	No	Yes (3)	No	No	No

^a Refer to the text for a discussion of each assay performance.

^b Bruker Biotyper v3.1 research-use-only (RUO) database.

^c Claimed only by Conformité Européenne for *in vitro* diagnostics (IVD-CE).

^d Indicates true and most current designation in the *S. bovis* group.

^e Labeled as *S. lutetiensis* in Bruker Biotyper v3.1 RUO.

^f Cleared by the U.S. Food and Drug Administration for *in vitro* diagnostics (IVD-FDA) and claimed by IVD-CE.

^g Labeled as *S. infantarius* in Bruker Biotyper v3.1 RUO.

discussed below. A summary of *S. bovis* group members that are listed by the manufacturer to be represented in each commercial test system is provided in Table 1. Importantly, no single test system can provide unequivocal identification, and molecular techniques are often used to complement phenotypic findings.

PHENOTYPIC AND BIOCHEMICAL METHODS

Until the early 2000s, *Streptococcus* species were identified primarily by hemolytic reactions, carbohydrate utilization, and phenotypic tests. Though the taxonomic history has been complicated, the *S. bovis* group has traditionally been described as Gram-positive cocci in pairs and short chains that were cultured as small gamma- or alpha-hemolytic colonies and that were catalase negative, leucine aminopeptidase positive, pyrrolidonyl arylamidase negative, bile tolerant, esculin hydrolysis positive, salt intolerant, and Lancefield group D positive (33). Further delineation into species and subspecies relied on the testing of β -glucuronidase, α - and β -galactosidase, β -mannosidase, and acid production from starch, glycogen, inulin, and mannitol (33).

Although phenotypic and biochemical tests remain the reference standards for identification within this group, recent studies (9, 22) have demonstrated that these traditional phenotypic and biochemical traits are not universal and can lead to potential misidentifications. In their study of 58 blood isolates, Beck et al. demonstrated that four major phenotypic characteristics that were traditionally used to define the *S. bovis* group and differentiate them from the enterococci (salt intolerance, bile tolerance, esculin hydrolysis, Lancefield group D typing) were in some instances unreliable (Table 2), emphasizing the significance of the pyrrolidonyl arylamidase test for separating the two groups (9). The variability in Lancefield group D agglutination using modern commercial test kits was further supported by Youn et al. who showed that only 40 of 51 (78%) *S. bovis* group isolates (as identified by molecular and phenotypic methods) typed as group D, while nine were untypeable and two typed as group A (34). These findings are significant given the wide adoption of Lancefield grouping in clinical laboratories, especially in settings where antigen grouping with commercial kits may be the only discriminatory test per-

formed for *Streptococcus* spp. depending on organism source and laboratory complexity. Inconsistencies in enzymatic activity and acid production have also been found in a few studies (7, 9, 22).

Several commercial panels are available for *Streptococcus* identification, but few studies have focused on the accuracy of identification within the *S. bovis* group specifically. The API 20 strep and Rapid ID 32 strep systems (bioMérieux, Marcy l'Etoile, France) are the most widely referenced panels in *S. bovis* group literature. Beck et al. found a 97 to 98% correlation with 16S rRNA gene sequencing for identifying *S. gallolyticus* subsp. *gallolyticus*, *S. gallolyticus* subsp. *pasteurianus*, and *S. infantarius* subsp. *coli* ($n = 58$) (9). In 2009 to 2010, however, both panels underwent reformulation and taxonomic database updates (bioMérieux package inserts), and performance of the updated system with reference molecular testing has not been well studied. In another study, Youn et al. compared the Vitek 2 GP ID card (bioMérieux) to *sodA* sequencing and found 75% agreement between the methods (34).

Therefore, while commercial phenotypic systems have streamlined workflow by grouping many biochemical tests into a single panel, they do not permit unambiguous identification, because it is difficult to build a flexible and reproducible system that will encompass the different and somewhat limited biochemical traits exhibited by different strains of the same species or subspecies.

TABLE 2 Summary from Beck et al. (9) demonstrating the unreliability of key biochemical reactions that were traditionally used to differentiate the *S. bovis* group from *Enterococcus* spp.

Organism	Salt intolerance (%)	Growth and hydrolysis on bile esculin agar (%)	Lancefield group D positive (%)
<i>S. gallolyticus</i> subsp. <i>gallolyticus</i>	72	100	72
<i>S. gallolyticus</i> subsp. <i>pasteurianus</i>	58	83	8
<i>S. infantarius</i> subsp. <i>coli</i>	88	35	59

Care therefore must be taken with final interpretation of the organism identification based on phenotypic and biochemical findings alone.

MALDI-TOF MS

Over the last 5 years, MALDI-TOF MS has transformed the clinical microbiology and infectious diseases fields, enabling significantly faster time to identification at a discriminatory power that is considerably higher than phenotypic and biochemical identification alone and similar to that of 16S rRNA gene sequencing (35). The clinical utility of this technology has been frequently reported; however, only a few studies have evaluated the performance of MALDI-TOF MS for accurate species and subspecies identification within the *S. bovis* group. By analyzing 52 previously identified *S. bovis* group isolates, Romero et al. reported that only 27 of 40 (68%) *S. gallolyticus* isolates were identified by the Biotyper database (Bruker Daltonics, Billerica, MA) to the species level, but none were identified to the subspecies level when a combination of 16S rRNA gene and *sodA* sequencing was used as the reference standards for identification (36). Identification accuracy was not addressed in this study, but the authors commented that subspecies-level identification at the time of study (2011) was not achievable with MALDI-TOF MS (36). These findings are in striking contrast with those of Hinse et al., who, working with a different system, demonstrated species- and subspecies-level discrimination based on dendrogram analysis of mass spectral profiles using the SARAMIS database (bioMérieux) with the AXIMA confidence MALDI-TOF MS instrument (Shimadzu, Japan), although the authors commented that *S. gallolyticus* subsp. *pasteurianus* could not always be separated from *S. gallolyticus* subsp. *gallolyticus* (37). These discrepant conclusions suggest that identification accuracy of MALDI-TOF MS is strongly dependent on technical details given the different instruments, spectral databases, and algorithms employed in the two studies (Table 1). To address this issue comprehensively, Youn et al. systematically analyzed 51 previously identified *S. bovis* group isolates with various protein extraction methods against two different MALDI-TOF MS instruments (Bruker Microflex and bioMérieux Vitek MS) and four different MALDI-TOF MS databases (Bruker Biotyper v3.1, Vitek MS v2.0, Vitek MS v3.0, and SARAMIS) using *sodA* sequencing and a general BLAST analysis of the NCBI database as the reference standard (34). In this study, the Vitek MS v3.0 system performed best, with 76 to 92% agreement with the results of *sodA* sequencing, depending on the protein extraction method used (34).

These studies demonstrate that MALDI-TOF MS can provide reasonably accurate species- and subspecies-level identification within the *S. bovis* group; however, the accuracy and discriminatory power of this technology is highly dependent upon the instrument, methods, and databases employed. Supplementation with biochemical and/or molecular testing may be needed for discrimination in some cases.

GENOMIC ANALYSIS

Over the years, numerous target genes, including *sodA*, 16S rRNA gene, *rpoB*, *groEL*, *gyrB*, and *recN*, have been analyzed for their discriminatory potential in providing species- and subspecies-level differentiation within the *S. bovis* group (13, 34, 38, 39). All such studies face similar challenges in defining reference identifications for the sets of isolates against which new molecular meth-

ods are tested. This has proved difficult and controversial in many cases, given the lack of an accepted reference molecular identification method and changing taxonomic definitions. Even though partial sequencing of the 16S rRNA gene is generally considered adequate for most routine clinical bacterial identification, analysis of six *S. bovis* group type strains showed a percent identity that was too close (97.1% to 99.8%) to differentiate species and subspecies within the group (13). In contrast, other authors have argued on the basis of comparative studies that partial sequencing of the *sodA* gene (~480 bp; 82% coverage) may provide better discriminatory power by dividing the *S. bovis* group into five main clusters (13, 38). More recently, a comprehensive study of 65 *Streptococcus* type strains found that partial sequencing of the *groEL* gene (757 bp, ~47% coverage) may be another useful target (39). Additional testing of *groEL* sequences on a larger subset of isolates will be useful to understand its utility relative to the other methods mentioned above.

A current major limitation is the lack of curated, well-populated, and updated sequence databases that can provide reliable organism identification. Several contradictory results have been published in the *S. bovis* group literature, with unreliable 16S rRNA gene and *sodA* sequences of type strains deposited in GenBank (9, 37). Many anticipate that use of new approaches, such as whole-genome sequencing, may result in another reevaluation of taxonomic status in the future (40). Keeping up to date with these changes and understanding their clinical relevance will be imperative for clinical microbiologists and infectious disease physicians alike.

ANTIMICROBIAL SUSCEPTIBILITY

Antimicrobial susceptibility data for the *S. bovis* group have remained relatively stable over the years, with MICs in the susceptible range reported for β -lactams (penicillin, ampicillin, amoxicillin-clavulanate, ceftriaxone, oxacillin, meropenem) and vancomycin; however, variable results have been observed for clindamycin, erythromycin, and levofloxacin (9, 34, 36). Surprisingly, a D test positivity rate of 89% for clindamycin-susceptible, erythromycin-resistant isolates was observed in one study (34), and all *S. bovis* group isolates in another study exhibited low-level resistance to the aminoglycosides (36). A high percentage of tetracycline resistance reported by Beck et al. (9) may be possibly explained by the recent identification of pSGG1, a novel plasmid carrying genes for tetracycline resistance that has strong sequence similarities to plasmids and chromosomes in several ruminal and gastrointestinal bacteria, thus suggesting a potential for horizontal gene transfer (40). Future genomic studies may highlight other potential mechanisms of resistance.

CONCLUSIONS

Species- and subspecies-level identification within the *S. bovis* group has become increasingly important as specific disease associations, including gastrointestinal neoplasms, meningitis, and endocarditis, are recognized. Reference identification in the *S. bovis* group still relies on biochemical and phenotypic-based assays; however, data show that single-gene-based molecular testing (16S rRNA gene and *sodA*) and MALDI-TOF MS may be useful in providing additional information to help enable species- and subspecies-level discrimination. When possible, both gene targets can be sequenced to increase confidence in identification accuracy; however, genomic sequencing is beyond the capabilities of many

clinical microbiology laboratories. Studies published to date suggest that the accuracy of MALDI-TOF MS is highly dependent on the system used and the databases employed. For these systems, the responsibility falls on the laboratory to validate and determine the accuracy of identifications provided by research-use-only (RUO) databases. In time, and with use of appropriately supplemented and updated databases, commercial platforms may be able to identify *S. bovis* group members at the species- and subspecies-level accurately and consistently. Table 1 illustrates the many differences observed between various commercial platforms, making it difficult to develop a simple and practical identification algorithm for microbiologists. Until further data are generated, many labs may choose to continue to report *S. bovis* at the group level, with exclusive reporting to the species or subspecies level performed only upon request and with careful consideration of the results obtained across multiple test methods and the clinical context of the patient. A thorough review of culture collection strains is warranted, because many isolates may have been originally misidentified and/or deposited before taxonomic revision. Care should also be taken when reviewing the literature due to changing nomenclature and the application of different methods for species and/or subspecies designation over time. It is envisaged that, once accurate identification models are in place, clinicians may be better equipped to predict potential disease associations and/or discover new organism-disease associations.

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