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Comprehensive Study of Strains Previously Designated *Streptococcus bovis* Consecutively Isolated from Human Blood Cultures and Emended Description of *Streptococcus gallolyticus* and *Streptococcus infantarius* subsp. *coli*[▽]

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Modern taxonomy has delineated *Streptococcus gallolyticus* subsp. *gallolyticus*, *S. gallolyticus* subsp. *pasteurianus*, *Streptococcus infantarius* subsp. *coli*, and *S. infantarius* subsp. *infantarius* within the heterogenous group of previously designated clinical *Streptococcus bovis* bacteria. In the present study, 58 consecutive blood culture isolates initially designated *S. bovis* were further characterized by applying phenotypic and molecular genetic methods, and possible disease associations were investigated by studying the patients' records. Published phenotypic characteristics of *S. gallolyticus* and *S. infantarius* were not unequivocal and did not allow an unambiguous phenotypic differentiation of the 58 clinical isolates. However, full-length 16S rRNA gene sequences clearly assigned the strains to *S. gallolyticus* subsp. *gallolyticus* ($n = 29$), *S. gallolyticus* subsp. *pasteurianus* ($n = 12$), and *S. infantarius* subsp. *coli* ($n = 17$). Only 28% of the patients with available records presented with endocarditis and 7% presented with colon carcinoma, whereas 37% of the patients had altered liver parenchyma and 28% had gall bladder disease as underlying diseases. Detailed antimicrobial susceptibility data on both *S. gallolyticus* subspecies and *S. infantarius* subsp. *coli* are given for the first time. As a result of the extensive characterization of the largest number of *S. gallolyticus* and *S. infantarius* human clinical isolates published so far, emended species descriptions are given. It is recommended that both clinical microbiologists and infectious disease specialists avoid the designation *S. bovis* for true *S. gallolyticus* and *S. infantarius* strains in the future in order to get a clearer picture of the possible disease associations of these species.

Since the late 1970s, clinical microbiologists and infectious disease specialists have had the engram of *Streptococcus bovis* endocarditis and the association of *S. bovis* and colon carcinoma whenever this taxon was isolated from human blood cultures (8). Traditionally, *S. bovis* had been divided into the three biotypes I (mannitol fermentation positive), II/1 (mannitol negative and β-glucuronidase negative), and II/2 (mannitol negative and β-glucuronidase positive) (3, 13). Since the mid-1980s, it was clear that *S. bovis* was a conglomerate of several genomospecies (4). During the late 1990s and at the beginning of this decade, the group of Bouvet, Grimont, and Schlegel demonstrated that the former *S. bovis* biotype I belongs to *Streptococcus gallolyticus* subsp. *gallolyticus* (*S. gallolyticus* had been defined by Osawa et al. in 1995 [11]), that the former biotype II/1 is, in fact, *Streptococcus infantarius* subsp. *coli* (intermittently designated *Streptococcus lutetiensis* by Poyart et al. [12]), and that biotype II/2 is *S. gallolyticus* subsp. *pasteurianus* (intermittently designated *S. pasteurianus* [12]) (14, 15). However, the identity of previously designated *S. bovis* isolated from human blood cultures has, so far, not been systematically investigated in the light of modern taxonomy. Therefore, two large private clinical laboratories from southern Germany decided to elucidate the identity of *S. bovis* human blood culture strains consecutively isolated in their routine laboratories. The possible disease associations of taxonomically correctly identified strains also should be investigated. As a result of a comprehensive study applying both phenotypic and molecular genetic methods, emended species descriptions of *S. gallolyticus* and *S. infantarius* subsp. *coli* are given.

MATERIALS AND METHODS

Study subjects. Patients' records were available in 46 of 58 (79%) of the cases studied and were received from the medical institutions in which the patients were hospitalized.

Strains. The type strains *S. gallolyticus* subsp. *gallolyticus* CCUG 35224^T, *S. gallolyticus* subsp. *pasteurianus* CCUG 46150^T, *S. infantarius* subsp. *coli* CCUG 47831^T, and *S. infantarius* subsp. *infantarius* CCUG 43820^T were received from the Culture Collection of the University of Göteborg (CCUG), Sweden. Strains with running numbers 1 to 34 and 55 to 58 (Table 1) were collected by Gärtner & Colleagues Laboratories, Ravensburg, Germany, and strains with running numbers 35 to 54 came from Limbach Laboratories, Heidelberg, Germany. Only one strain per patient was included in the present study. All 58 clinical strains were recovered from BACTEC blood culture bottles (BD, Heidelberg, Germany), which had been incubated for less than 3 days. Subcultures were grown on Columbia base sheep blood agar plates (SBA) (BD) after incubation for 20 to 24 h at 35°C in a 5% CO_2-enriched atmosphere. Strains were stored in skim milk at −20°C until further use; the recovery of the strains was, again, on SBA.

Biochemical profiles and antigen detection. The commercial API 20 Strep and the Rapid ID 32 Strep identification systems (both from bioMérieux, Marcy l'Etoile, France) were used according to the manufacturer's instructions, and reading was done by two independent researchers. Salt tolerance was examined in 6.5% NaCl nutrient broth (Heipha, Eppelheim, Germany) after incubating the tubes for 24 h at 35°C. The ability to grow (regardless of the esculin reaction) on bile esculin agar (BD) was tested by reading the plates after 48 h of incubation

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TABLE 1. Consecutive human blood culture isolates tentatively identified as *S. bovis* and included in the present study

Running no.	Internal strain no.	Patient's sex, age[a]	Mo/yr of isolation	Town of patient's hospitalization	Identification	GenBank accession no.
1	621	F, 77	11/2004	Heidenheim	*S. gallolyticus* subsp. *pasteurianus*	EU163439
2	628	F, 71	12/2004	Villingen	*S. gallolyticus* subsp. *pasteurianus*	EU163440
3	629	F, 54	12/2004	Balingen	*S. gallolyticus* subsp. *gallolyticus*	EU163441
4	630	M, 76	12/2004	Villingen	*S. gallolyticus* subsp. *gallolyticus*	EU163442
5	633	F, 83	12/2004	Ehingen	*S. infantarius* subsp. *coli*	EU163443
6	644	M, 74	12/2004	Günzburg	*S. infantarius* subsp. *coli*	EU163444
7	647	M, 55	2/2005	Kaufbeuren	*S. infantarius* subsp. *coli*	EU163445
8	652	M, 68	2/2005	Neu-Ulm	*S. gallolyticus* subsp. *gallolyticus*	EU163447
9	660	M, 71	3/2005	Ottobeuren	*S. gallolyticus* subsp. *gallolyticus*	EU163448
10	668	M, 65	3/2005	Aalen	*S. gallolyticus* subsp. *pasteurianus*	EU163449
11	693	M, 66	5/2005	Rottweil	*S. gallolyticus* subsp. *pasteurianus*	EU163451
12	695	M, 69	6/2005	Friedrichshafen	*S. infantarius* subsp. *coli*	EU163452
13	702	M, 75	6/2005	Friedrichshafen	*S. infantarius* subsp. *coli*	EU163453
14	706	M, 67	6/2005	Ravensburg	*S. gallolyticus* subsp. *gallolyticus*	EU163454
15	710	M, 74	8/2005	Günzburg	*S. infantarius* subsp. *coli*	EU163455
16	712	F, 84	8/2005	Heidenheim	*S. gallolyticus* subsp. *gallolyticus*	EU163456
17	713	M, 66	8/2005	Kempten	*S. gallolyticus* subsp. *gallolyticus*	EU163457
18	718	F, 72	8/2005	Schwenningen	*S. gallolyticus* subsp. *gallolyticus*	EU163458
19	719	M, 72	8/2005	Ravensburg	*S. gallolyticus* subsp. *gallolyticus*	EU163459
20	721	F, 77	9/2005	Heidenheim	*S. gallolyticus* subsp. *pasteurianus*	EU163460
21	724	M, 65	9/2005	Ottobeuren	*S. gallolyticus* subsp. *gallolyticus*	EU163461
22	746	M, 74	10/2005	Rottweil	*S. gallolyticus* subsp. *gallolyticus*	EU163462
23	749	M, 42	10/2005	Immenstadt	*S. gallolyticus* subsp. *pasteurianus*	EU163463
24	750	M, 67	10/2005	Kaufbeuren	*S. gallolyticus* subsp. *gallolyticus*	EU163464
25	757	M, 73	11/2005	Ravensburg	*S. gallolyticus* subsp. *pasteurianus*	EU163465
26	760	F, 74	11/2005	Heidenheim	*S. infantarius* subsp. *coli*	EU163466
27	762	M, 62	12/2005	Kempten	*S. infantarius* subsp. *coli*	EU163467
28	773	F, 89	1/2006	Crailsheim	*S. infantarius* subsp. *coli*	EU163469
29	790	M, 51	3/2006	Mindelheim	*S. infantarius* subsp. *coli*	EU163470
30	816	M, 63	6/2006	Neu-Ulm	*S. gallolyticus* subsp. *gallolyticus*	EU163471
31	825	M, 43	7/2006	Wangen	*S. gallolyticus* subsp. *gallolyticus*	EU163472
32	826	M, 50	7/2006	Donaueschingen	*S. gallolyticus* subsp. *gallolyticus*	EU163473
33	837	M, 44	8/2006	Friedrichshafen	*S. gallolyticus* subsp. *gallolyticus*	EU163474
34	838	F, 57	8/2006	Crailsheim	*S. gallolyticus* subsp. *pasteurianus*	EU163475
35	841	M, 73	7/2005	Troisdorf	*S. gallolyticus* subsp. *gallolyticus*	EU163476
36	842	M, 66	8/2005	Koblenz	*S. gallolyticus* subsp. *gallolyticus*	EU163477
37	843	M, 81	9/2005	Heidelberg	*S. infantarius* subsp. *coli*	EU163478
38	844	M, 76	10/2005	Erbach	*S. infantarius* subsp. *coli*	EU163479
39	845	F, 61	10/2005	Gelsenkirchen	*S. infantarius* subsp. *coli*	EU163480
40	846	M, 72	11/2005	Duisburg	*S. gallolyticus* subsp. *gallolyticus*	EU163481
41	847	F, 68	11/2005	Bonn	*S. gallolyticus* subsp. *gallolyticus*	EU163482
42	848	M, 56	1/2006	Gelsenkirchen	*S. gallolyticus* subsp. *pasteurianus*	EU163483
43	849	M, 80	1/2006	Nürnberg	*S. gallolyticus* subsp. *gallolyticus*	EU163484
44	850	M, 77	1/2006	Daun	*S. gallolyticus* subsp. *gallolyticus*	EU163485
45	851	F, 57	2/2006	Speyer	*S. gallolyticus* subsp. *gallolyticus*	EU163486
46	852	F, 62	3/2006	Heidelberg	*S. gallolyticus* subsp. *gallolyticus*	EU163487
47	853	M, 70	3/2006	Erbach	*S. gallolyticus* subsp. *gallolyticus*	EU163488
48	854	M, 60	5/2006	Bonn	*S. infantarius* subsp. *coli*	EU163489
49	855	F, 75	5/2006	Sinsheim	*S. gallolyticus* subsp. *gallolyticus*	EU163490
50	856	F, 90	6/2006	Bonn	*S. infantarius* subsp. *coli*	EU163491
51	857	M, 81	7/2006	Erbach	*S. infantarius* subsp. *coli*	EU163492
52	858	F, 81	7/2006	Koblenz	*S. gallolyticus* subsp. *pasteurianus*	EU163493
53	859	M, 70	7/2006	Heidelberg	*S. gallolyticus* subsp. *gallolyticus*	EU163494
54	860	M, 80	8/2006	Neustadt	*S. gallolyticus* subsp. *pasteurianus*	EU163495
55	863	F, 77	8/2006	Schwäbisch-Gmünd	*S. gallolyticus* subsp. *pasteurianus*	EU163496
56	864	M, 73	8/2006	Krumbach	*S. infantarius* subsp. *coli*	EU163497
57	865	M, 72	8/2006	Mindelheim	*S. gallolyticus* subsp. *gallolyticus*	EU163498
58	868	M, 59	9/2006	Aalen	*S. gallolyticus* subsp. *gallolyticus*	EU163499

[a] F, female; M, male.

at 35°C. For the detection of the Lancefield group D antigen, a commercial antiserum was used (Oxoid, Basingstoke, United Kingdom).

Antimicrobial susceptibility testing. For the determination of MICs, all clinical strains included in the present study were tested by a microdilution method (cation-adjusted Mueller-Hinton broth with lysed horse blood) according to the published CLSI standard (1).

Molecular genetic investigations. The analysis of the complete 16S rRNA gene sequences was performed according to a published protocol with broad-range primers TPU-1 (AGAGTTTGATCMTGGCTCAG) and RTU-8 (AAGGAGG TGATCCAKCCRCA) (5). The following primers were used for cycle sequencing: TPU-1, TPU-2 (CCARACTCCTACGGGAGGCA), TPU-3 (CAGCMGC CGCGGTAATWC), TPU-4 (GGATTAGATACCCTGGTAGTCC), TPU-5 (A

AACTYAAAKGAATTGACGG), TPU-6 (GGGCKACACACGTGCTACA AT), TPU-7 (GAATACGTTCCCGGGYCTTGT), RTU-2 (TGCCTCCCGTA GGAGTYTGG), RTU-3 (GWATTACCGCGGCKGCTG), RTU-4 (TACCAG GGTATCTAATCCTGTT), RTU-5 (CCGTCAATTCMTTTRAGTTT), RTU-6 (ATTGTAGCACGTGTGTMGCCC), RTU-7 (ACAAGRCCCGGGAACGTA TT), and RTU-8. The DNA strand was sequenced in both directions, and the full-length sequences were determined by aligning multiple overlapping DNA sequences with the Lasergene 5 package (DNASTAR Inc., Madison, WI).

Strains deposited. The following strains with unusual biochemical reactions have been deposited in the CCUG: strain 668 *S. gallolyticus* subsp. *pasteurianus* (β-glucuronidase negative) as CCUG 55345, strain 749 *S. gallolyticus* subsp. *pasteurianus* (β-galactosidase negative) as CCUG 55346, strain 773 *S. infantarius* subsp. *coli* (α-galactosidase negative) as CCUG 55347, strain 825 *S. gallolyticus* subsp. *gallolyticus* (β-glucosidase negative) as CCUG 55348, strain 848 *S. gallolyticus* subsp. *pasteurianus* (β-mannosidase negative) as CCUG 55349, strain 858 *S. gallolyticus* subsp. *pasteurianus* (β-galactosidase negative) as CCUG 55350, and strain 860 *S. gallolyticus* subsp. *pasteurianus* (β-mannosidase negative) as CCUG 55351.

Nucleotide sequence accession numbers. The GenBank accession numbers of the complete 16S rRNA gene sequences of all 58 clinical isolates included in the present study are given in Table 1. The 16S rRNA gene sequences of the type strains of *S. gallolyticus* subsp. *gallolyticus*, *S. gallolyticus* subsp. *pasteurianus*, and *S. infantarius* subsp. *coli* have been deposited in GenBank under accession numbers EU163500, EU163502, and EU163503.

RESULTS

During a period of 23 months, Gärtner & Colleagues Laboratories collected 38 *S. bovis* isolates (Table 1); Limbach Laboratories collected 20 *S. bovis* strains during a 13-month period. The 58 isolates came from 33 different hospitals in which the patients were treated and which were located mainly in the southwestern part of Germany. Table 1 also gives the biographical data of all 58 patients included in the present study. Forty (69%) of the 58 patients were male, and 18 (31%) were female. The mean age of the male patients was 66.8 ± 10.3 years and was similar for all three bacterial taxa groups detected; the mean age for the female patients was 72.7 ± 10.7 years. Of all 228 blood culture bottles drawn from the 58 patients, 86% were positive for *S. bovis*. In 76% of the 58 cases, all blood cultures received from an individual patient became positive for *S. bovis*.

When applying 16S rRNA gene sequencing, 29 (50%) of all *S. bovis* strains finally were identified as *S. gallolyticus* subsp. *gallolyticus*, 12 strains (21%) as *S. gallolyticus* subsp. *pasteurianus*, and 17 strains (29%) as *S. infantarius* subsp. *coli*, whereas not a single strain of *S. infantarius* subsp. *infantarius* was detected.

Table 2 outlines the most relevant clinical data of the patients. Interestingly, nearly two-thirds of the patients had a hepatobiliary disorder as the underlying disease. The following comorbidities were detected in the 46 patients with available patients' records: cardiovascular disease, 80%; diabetes mellitus, 35%; other malignancies, 22%; kidney disease, 15%; and respiratory disease, 13%. Endocarditis was present in less than one-third of all patients, and colon carcinoma was detected in less than 10% of all patients with *S. bovis* bacteremia. For 11 of the 46 patients, stool was screened for occult blood, but only 2 of 11 patients were positive. For 15 of the 46 patients colonoscopy was performed, but only two cases of colon carcinoma were detected. Six (13%) of the 46 patients died during the hospitalization in which *S. bovis* bacteremia occurred, but in only 2 of the 6 cases was *S. bovis* bacteremia the single cause leading to the patient's death.

TABLE 2. Main clinical data of patients included in the present study

Feature	No. (%[b]) of strains exhibiting feature		
	S. gallolyticus subsp. *gallolyticus* (n = 21[a])	*S. gallolyticus* subsp. *pasteurianus* (n = 11)	*S. infantarius* subsp. *coli* (n = 14)
Underlying disease			
Altered liver parenchyma	8 (38)	3 (27)	6 (43)
Gall bladder disease	5 (24)	4 (36)	4 (29)
Benign colon disease	5 (24)	0 (0)	3 (21)
Clinical presentation			
Endocarditis present	9 (43)	0 (0)	4 (29)
Colon carcinoma present	2 (10)	0 (0)	1 (7)

[a] Number of patients for whom records were available.
[b] Percentage of patients positive for the given feature out of those for whom records were available.

Whenever small-colony, catalase-negative, gram-positive cocci are isolated in pure culture from blood cultures, our routine laboratories presently apply the commercial API 20 Strep and the Rapid ID 32 Strep systems for identification. All 29 *S. gallolyticus* subsp. *gallolyticus* strains were identified as *S. bovis* biotype I by both identification systems. Eleven of the 12 *S. gallolyticus* subsp. *pasteurianus* strains were identified as *S. bovis* II/2 (one strains was identified as *Streptococcus mutans*) by the API 20 Strep system and all 12 strains by the Rapid ID 32 Strep system. All 17 *S. infantarius* subsp. *coli* strains were identified as *S. bovis* II/1 by the API 20 Strep system and 15 out of 17 strains by the Rapid ID 32 Strep system (the two other strains were identified as *Aerococcus viridans* and *Leuconostoc* spp., respectively).

Particular biochemical reactions (Table 3) of *S. gallolyticus* and *S. infantarius* subsp. *coli* are not always clear-cut, i.e., either 0 or 100% positive. As given in Table 3, reactions differed in the two different identification systems used due to different substrates or pH conditions in the commercial devices. Eight of the 29 (28%) *S. gallolyticus* subsp. *gallolyticus* strains, 5 of the 12 (42%) *S. gallolyticus* subsp. *pasteurianus* strains, and 2 of the 17 (12%) *S. infantarius* subsp. *coli* strains grew in 6.5% NaCl. All 29 *S. gallolyticus* subsp. *gallolyticus* strains grew on bile esculin agar, whereas 2 of the 12 (17%) *S. gallolyticus* subsp. *pasteurianus* strains and 11 of the 17 (65%) *S. infantarius* subsp. *coli* strains were unable to grow on bile esculin agar. Finally, 21 of the 29 (72%) *S. gallolyticus* subsp. *gallolyticus* strains reacted with Lancefield group D antiserum, but only 1 of the 12 (8%) *S. gallolyticus* subsp. *pasteurianus* strains and 10 of the 17 (59%) *S. infantarius* subsp. *coli* strains expressed the Lancefield group D antigen.

Table 4 shows the antimicrobial susceptibility patterns of *S. gallolyticus* and *S. infantarius* subsp. *coli*. All 58 clinical isolates were susceptible to β-lactams and vancomycin. Variable susceptibility to erythromycin was detected mainly in *S. gallolyticus*, with MICs being either low (e.g., 0.06 μg/ml) or high (e.g., >32 μg/ml). For nearly all 58 clinical isolates, levofloxacin susceptibility fell into the intermediate susceptibility category. The majority of the isolates were resistant to tetracycline. There was a tendency of *S. infantarius* subsp. *coli* isolates to be

TABLE 3. Biochemical reactions of *S. gallolyticus* subsp. *gallolyticus*, *S. gallolyticus* subsp. *pasteurianus*, and *S. infantarius* subsp. *coli*

Biochemical reaction	Reaction[a] for:		
	S. gallolyticus subsp. *gallolyticus* (n = 29)	*S. gallolyticus* subsp. *pasteurianus* (n = 12)	*S. infantarius* subsp. *coli* (n = 17)
Esculin hydrolysis	100	100	41
Enzymatic activity			
β-Glucuronidase	0	92	0
	0	92	0
α-Galactosidase	7	92	100
	3	83	94
β-Galactosidase	3	83	0
	10	83	0
β-Mannosidase	14	83	0
Acid production			
Starch	100	8	100
Glycogen	100	0	71
	100	0	94
Inulin	55	0	0
Mannitol	93	0	0
	93	8	0
Methyl-β-D-gluco-pyranoside	100	92	29
Raffinose	97	92	47
	100	92	100
Trehalose	100	100	12
	100	100	12

[a] Values are the percentages of strains positive for the indicated reaction. If two percentages per reaction are given, the upper number represents the data derived from using the Rapid ID 32 Strep identification system and the lower number the data from using the API 20 STREP identification system.

more susceptible to antimicrobial agents than *S. gallolyticus* strains.

For comparative 16S rRNA gene analysis, we first sequenced the four type strains of *S. gallolyticus* (two subspecies) and *S. infantarius* (two subspecies). All 58 clinical isolates were compared to those derived from four type strain sequences, which have been deposited by us in GenBank as EU163500 (*S. gallolyticus* subsp. *gallolyticus*), EU163502 (*S. gallolyticus* subsp. *pasteurianus*), EU163503 (*S. infantarius* subsp. *coli*), and EU163504 (*S. infantarius* subsp. *infantarius*). The 16S rRNA gene sequences of the type strains available from the GenBank/EMBL database had been found to be unreliable, with, e.g., S. *gallolyticus* subsp. *gallolyticus*^{T} (X94337), showing 34-bp differences (including 12 N residues) from the true sequence.

The 16S rRNA gene sequences of the strains belonging to a particular taxon were extremely highly conserved (Table 5). Twenty-six of 29 *S. gallolyticus* subsp. *gallolyticus* strains showed identical 16S rRNA gene sequences (1,453 bp), and only strain 837 had a single point mutation (T instead of C at position 970) as well as strains 847 and 849 at position 1106 (G instead of C). Similar results were observed for *S. gallolyticus* subsp. *pasteurianus*, with 11 of 12 strains showing identical sequences (i.e., 100% homology for 1,197 bp), and only strain 757 had a single point mutation within the 16S rRNA gene (A instead of G at position 1154). Regarding the 17 *S. infantarius* subsp. *coli* strains, only strain 644 showed a single point mutation (T instead of C at position 980), whereas the other 16 *S. infantarius* subsp. *coli* strains exhibited absolutely identical sequences (1,321 bp). The 16S rRNA genes of *S. infantarius* subsp. *coli* strains were constantly different from *S. infantarius*

TABLE 4. Antimicrobial susceptibility patterns of *S. gallolyticus* subsp. *gallolyticus*, *S. gallolyticus* subsp. *pasteurianus*, and *S. infantarius* subsp. *coli*

Species and antimicrobial agent	MIC (μg/ml)			Susceptibility category[a]		
	Range	50%	90%	Susceptible	Intermediate	Resistant
S. gallolyticus subsp. *gallolyticus* (n = 29)						
Ceftriaxone	0.12–0.25	0.25	0.25	29/29 (100)	0/29 (0)	0/29 (0)
Erythromycin	0.03–>32	0.12	>32	17/29 (59)	1/29 (3)	11/29 (38)
Levofloxacin	1–8	2	4	2/29 (7)	27/29 (93)	0/29 (0)
Meropenem	≤0.03	≤0.03	≤0.03	29/29 (100)	0/29 (0)	0/29 (0)
Penicillin	0.06–0.12	0.06	0.12	29/29 (100)	0/29 (0)	0/29 (0)
Tetracycline	0.5–>64	64	>64	9/29 (31)	0/29 (0)	20/29 (69)
Vancomycin	0.25–0.5	0.5	0.5	29/29 (100)	0/29 (0)	0/29 (0)
S. gallolyticus subsp. *pasteurianus* (n = 12)						
Ceftriaxone	0.12–0.25	0.25	0.25	12/12 (100)	0/12 (0)	0/12 (0)
Erythromycin	0.06–>32	0.12	>32	8/12 (67)	0/12 (0)	4/12 (33)
Levofloxacin	2–>64	4	8	0/12 (0)	11/12 (92)	1/12 (8)
Meropenem	≤0.03–0.06	≤0.03	≤0.03	12/12 (100)	0/12 (0)	0/12 (0)
Penicillin	0.06–0.12	0.06	0.12	12/12 (100)	0/12 (0)	0/12 (0)
Tetracycline	0.25–>64	64	>64	2/12 (17)	0/12 (0)	10/12 (83)
Vancomycin	0.25–0.5	0.5	0.5	12/12 (100)	0/12 (0)	0/12 (0)
S. infantarius subsp. *coli* (n = 17)						
Ceftriaxone	≤0.03–0.25	0.25	0.25	17/17 (100)	0/17 (0)	0/17 (0)
Erythromycin	0.06–>32	0.06	0.12	16/17 (94)	0/17 (0)	1/17 (6)
Levofloxacin	1–>32	2	32	1/17 (6)	14/17 (82)	2/17 (12)
Meropenem	≤0.03–0.06	≤0.03	0.06	17/17 (100)	0/17 (0)	0/17 (0)
Penicillin	0.06–0.12	0.12	0.12	17/17 (100)	0/17 (0)	0/17 (0)
Tetracycline	0.25–>64	8	>64	8/17 (47)	1/17 (6)	8/17 (47)
Vancomycin	0.25–0.5	0.5	0.5	17/17 (100)	0/17 (0)	0/17 (0)

[a] Values are the numbers of isolates belonging to a certain category; percentages are in parentheses.

TABLE 5. 16S rRNA gene data allowing a unanimous differentiation of *S. gallolyticus* subsp. *gallolyticus*, *S. gallolyticus* subsp. *pasteurianus*, and *S. infantarius* subsp. *coli*

Nucleotide position	Nucleotide (no. of strains in which the particular nucleotide is present/no. of all strains analyzed) in:		
	S. gallolyticus subsp. *gallolyticus*[a]	*S. gallolyticus* subsp. *pasteurianus*[b]	*S. infantarius* subsp. *coli*[c]
36	C (28/28)	C (11/11)	A (15/15)
37	T (28/28)	T (11/11)	G (15/15)
52	G (29/29)	G (11/11)	A (16/16)
56	A (29/29)	A (11/11)	T (16/16)
58	A (29/29)	A (11/11)	G (16/16)
60	G (29/29)	G (11/11)	A (16/16)
148	T (29/29)	T (11/11)	C (16/16)
149	G (29/29)	G (11/11)	A (16/16)
156	A (29/29)	A (11/11)	C (16/16)
176	A (29/29)	A (11/11)	G (17/17)
177	T (29/29)	T (11/11)	A (17/17)
182	A (29/29)	T (11/11)	T (17/17)
186	A (29/29)	A (11/11)	T (17/17)
227	G (29/29)	G (11/11)	A (17/17)
237	C (29/29)	C (11/11)	T (17/17)
238	T (29/29)	C (11/11)	C (17/17)
618	A (29/29)	A (12/12)	G (17/17)
980	T (29/29)	T (12/12)	C (16/17), T (1/17)
991	A (29/29)	A (12/12)	G (17/17)
1106	C (27/29), G (2/29)	T (12/12)	A (17/17)
1229	A (29/29)	A (12/12)	G (17/17)
1419	T (29/29)	A (12/12)	T (17/17)

[a] The determined 16S rRNA gene sequence of strain 660 did not include base pairs 36 and 37.
[b] The determined 16S rRNA gene sequence of strain 621 did not include base pairs 36 to 238.
[c] The determined 16S rRNA gene sequence of strain 843 did not include base pairs 36 to 156, and that of strain 864 did not include base pairs 36 and 37.

subsp. *infantarius* at positions 34 (G instead of A), 54 (G instead of T), and 419 (C instead of T).

The separation of the two subspecies of *S. gallolyticus* by means of 16S rRNA gene sequences is possible at four different nucleotide positions, namely 182, 238, 1106, and 1419 (Table 5), whereas both *S. gallolyticus* subspecies and *S. infantarius* subsp. *coli* differ at 19 nucleotide positions (Table 5). In addition, *S. gallolyticus* subsp. *gallolyticus* has two other mismatches with *S. infantarius* subsp. *coli* (positions 182 and 238), and *S. gallolyticus* subsp. *pasteurianus* has another individual mismatch with *S. infantarius* subsp. *coli* at position 1419 (Table 5).

DISCUSSION

To the best of our knowledge, the present study is the largest on consecutively isolated *S. bovis* blood culture isolates that applies appropriate nomenclature.

In our study, more male patients than females with *S. bovis* blood culture isolates were seen, as already reported in other studies (range, 66 to 82%) (2, 6, 18). The mean age of the patients in our series was also similar to that of the other studies (range, 59 to 67 years) (2, 6, 18). In contrast to most other studies on *S. bovis* blood culture isolates, we observed significantly fewer cases of endocarditis (29%) and colon carcinomas (7%) in all patients, whereas in the classic study by Ruoff et al., 94% of all bacteremic patients with *S. bovis* biotype I had endocarditis and 71% had colon carcinoma (13). A more recent study found that *S. bovis* bacteremia in 62 of 64 cases was clinically significant and *S. bovis* biotype I was associated with endocarditis or colon carcinoma in 74 or 57% of all cases, respectively (2). In the present study, colonoscopy was performed on 15 of 46 patients only. It is therefore not unlikely that some colon carcinomas have been missed. The reason for the overall lower percentage of endocarditis and colon carcinoma in our series is unclear but may indicate, at least in some geographic regions and at certain periods of time, that the association of *S. bovis* bacteremia and endocarditis and/or colon carcinoma is not as strong as previously thought. However, the association of *S. bovis* blood culture isolates and colon carcinoma in 7% of our present cases is still much higher than that for any other microorganism except for *Clostridium septicum*, which is associated with colon carcinoma in one-third of all cases with blood cultures positive for this particular bacterium (9).

Interestingly, we observed that more than 60% of our patients had a hepatobiliary disorder as the underlying disease. Corredoira et al. (2) reported that 50% of their *S. bovis* bacteremia patients had liver or gall bladder disease, but this was seen mainly in *S. bovis* biotype II patients (i.e., either *S. gallolyticus* subsp. *pasteurianus* or *S. infantarius* subsp. *coli*), whereas we also found these underlying diseases in patients with *S. gallolyticus* subsp. *gallolyticus*.

We did not detect a single strain of *S. infantarius* subsp. *infantarius* in our series, whereas Schlegel et al. (15) reported on some clinical isolates. However, Schlegel et al. (15) also included a strain from dairy products and one from frozen vegetables, indicating that this subspecies also can be isolated from food products or, even more often, from these products than from bacteremia cases of humans.

The clinical microbiologist often is confronted with a differential diagnosis between *S. bovis* isolates and enterococci when greyish, catalase-negative, gram-positive cocci are isolated from blood cultures. In our experience, *S. bovis* usually exhibits smaller colonies than enterococci. It is important that, in our series, 15 of 58 (26%) *S. bovis* strains grew in 6.5% NaCl broth, which is in contrast to a statement in a reference text book (16), so that this characteristic does not allow an unambiguous differentiation between enterococci (consistently salt tolerant) and *S. bovis*. Interestingly, we observed that all *S. gallolyticus* subsp. *gallolyticus* strains grew on bile esculin agar but that some *S. gallolyticus* subsp. *pasteurianus* strains and the majority of *S. infantarius* subsp. *coli* strains were unable to grow on this medium, which is, again, in contrast to the information provided in a major textbook (16). Schlegel et al. (14, 15) stated that their *S. bovis* isolates variably (e.g., 44% in *S. infantarius* subsp. *coli*) expressed the Lancefield group D antigen, and in our series 55% of all 58 *S. bovis* strains were positive for this reaction. Of note is that significantly fewer *S. gallolyticus* subsp. *pasteurianus* strains were expressing the Lancefield group D antigen than *S. gallolyticus* subsp. *gallolyticus* strains. Therefore, studies (2, 6) including only Lancefield group D streptococci isolated from blood cultures may have a bias toward *S. gallolyticus* subsp. *gallolyticus* and underestimate the number of *S. gallolyticus* subsp. *pasteurianus* strains. In summary, the traditional tests for enterococci (salt tolerance, growth on bile esculin agar, and expression of Lancefield group D antigen) do not allow a clear-cut distinction between enterococci and *S.*

bovis in every case, so that further biochemical testing, like that for the presence of pyrrolidonyl arylamidase (positive in enterococci but negative in *S. bovis*) and other reactions (Table 3) by means of commercial identification systems is required.

In the initial species description of *S. infantarius* subsp. *coli* by Schlegel et al., the authors did not define a type strain but rather defined a reference strain (15). The authors designated this particular strain CCUG 43822^T (14), whereas CCUG 47831^T would have been the correct CCUG strain number (E. Falsen, CCUG, personal communication). The use of the term reference strain instead of type strain for the species-defining strain of *S. infantarius* subsp. *coli* (15) has created some confusion, since the later publication of Poyart et al. (12) exactly described the same taxon using the species designation *S. lutetiensis*. The Judicial Commission of the International Committee on Systematic Bacteriology discussed the above issue at a recent meeting but did not come up with a final judgment (http://www.bacterio.cict.fr/s/streptococcus). Therefore, we decided to use the designation *S. infantarius* subsp. *coli* rather than either *S. lutetiensis* or *S. infantarius* subsp. *lutetiensis* in the present study. However, apart from this formal point, we found that some features of *S. infantarius* subsp. *coli* were different from the data given in references (14, 15): the authors stated that 100% of *S. infantarius* subsp. *coli* strains hydrolyze esculin, whereas we only found 41% of the strains do so. Furthermore, the authors described *S. infantarius* subsp. *coli* being unable to produce acid from either starch or glycogen, whereas in our study the acidification of starch was constantly (i.e., 100%) positive, and the majority of *S. infantarius* subsp. *coli* strains also produced acid from glycogen. Finally, Schlegel et al. (15) did not detect acid production from trehalose, whereas we found 2 of 17 strains to be positive for this reaction. These obviously differing characteristics, together with the largest number of *S. infantarius* subsp. *coli* strains published so far, allow us to give an emended species description for *S. infantarius* subsp. *coli* (see below).

Trehalose fermentation was described as being variable for *S. gallolyticus* subsp. *gallolyticus* (14), whereas we found that 100% of all strains were positive for this reaction. Acid production from starch was reported as variable for *S. gallolyticus* subsp. *pasteurianus*, whereas we found that only 1 of 12 strains was positive for this reaction. Schlegel et al. (14) observed all *S. gallolyticus* subsp. *pasteurianus* strains to exhibit activities of β-glucuronidase, β-galactosidase, and β-mannosidase, whereas we found some strains (which have been deposited in CCUG) not expressing these enzymes. Again, the partial differences between our data and the data of Schlegel et al. (14) allow an emended species description of *S. gallolyticus* (see below).

Susceptibility data on *S. gallolyticus* subsp. *pasteurianus* and *S. infantarius* subsp. *coli* have not been reported in the literature before. We did not observe any significant differences in the susceptibility pattern of the three taxa reported here; there was only a tendency of *S. infantarius* subsp. *coli* to be more susceptible to erythromycin and tetracycline than *S. gallolyticus*. Our susceptibility data are in agreement with older data from Thornsberry et al. (17) that show *S. bovis* being uniformly susceptible to β-lactams. Thornsberry et al. did not detect erythromycin resistance in their *S. bovis* isolates from the 1970s (17). Recent susceptibility data on *S. gallolyticus*, neither divided into the two subspecies nor clearly differentiated from *S. infantarius* subsp. *coli*, showed the same high level of tetracycline resistance (i.e., 66%) as that of the isolates in our study (10).

The present study gives by far the most detailed molecular genetic data on *S. gallolyticus* and *S. infantarius* subsp. *coli*. Schlegel et al. did not include detailed data on the 16S rRNA gene nucleotide positions of either *S. gallolyticus* or *S. infantarius* in their publications (14, 15). The previously largest series on *S. bovis* 16S rRNA gene sequences came from the Mayo Clinic, comprising 13 isolates of *S. gallolyticus* (7).

S. gallolyticus and *S. infantarius* subsp. *coli* share less than 99.0% 16S rRNA gene homology. With the data given in Table 5, probes or restriction fragment length polymorphism tests could be developed for species or subspecies identification because of the extremely low base pair variability within one species or subspecies.

Because the majority of clinical microbiologists use commercial devices for the identification of catalase-negative gram-positive cocci in their routine laboratory, it is recommended that the manufacturers of such devices rapidly improve the databases and implement the new taxonomy in their databases. The improved commercial products should not contain the summarizing species designation *S. bovis* for human isolates anymore, thereby enabling clinical microbiologists and infectious disease specialists to use the proper taxonomic species designations in order to get a clearer picture of possible disease associations of either *S. gallolyticus* or *S. infantarius* subsp. *coli*.

Based on the results of the studies of Schlegel et al. (14, 15) and our data presented here, we provide emended descriptions, including the GenBank 16S rRNA gene sequence accession numbers of both *S. gallolyticus* subspecies and *S. infantarius* subsp. *coli*.

Emended description of *Streptococcus gallolyticus* subsp. *gallolyticus* Schlegel et al. (2003), corr. Beck, Frodl, and Funke 2008. *Streptococcus gallolyticus* subsp. *gallolyticus* (gal.lo.ly'ticus. N.L. n. *gallatum* gallate; N.L. adj. *lyticus* able to loosen; N.L. adj. *gallolyticus* gallate digesting).

Strains hydrolyze methyl gallate (tannase activity), decarboxylate gallic acid to pyrogallol, and grow on bile esculin agar. They produce acid from starch, glycogen, methyl-β-D-glucopyranoside, and trehalose. Nearly all strains ferment mannitol and raffinose; most strains ferment inulin. Strains have been isolated from the feces of marsupials, such as koalas, kangaroos, brushtails, and possums, as well as from various mammals, such as cows, horses, pigs, dogs, and guinea pigs; some strains have been isolated from the sheep rumen, and some were shown to be responsible for bovine mastitis. Human strains can be isolated from blood or feces. The type strain is ACM 3611^T (= CCUG 35224^T = CIP 105428^T = JCM 10005^T = LMG 16802^T = HDP 98035^T). The 16S rRNA gene sequence of the type strain has been deposited in GenBank under accession number EU163500.

Emended description of *Streptococcus gallolyticus* subsp. *pasteurianus* Schlegel et al. (2003), corr. Beck, Frodl, and Funke 2008. *Streptococcus gallolyticus* subsp. *pasteurianus* (pas.teurí.a.nus N.L. masc. adj. *pasteurianus* of Pasteur, referring to the Pasteur Institute, where the type strain was characterized).

Strains produce β-glucosidase. The majority of strains, but not all, are positive for the activity of β-glucuronidase, β-man-

nosidase, α-galactosidase, and β-galactosidase. The acid production from trehalose, raffinose, methyl-β-D-glucopyranoside, melibiose, and melezitose is variable. Very few strains produce acid from starch or mannitol. The production of acid from glycogen and inulin is absent. Strains do not produce tannase, but some strains may yield gallate decarboxylase activity (11). Strains have been isolated from various human clinical sources. The type strain is NEM 1202^T (= CIP 107122^T). The 16S rRNA gene sequence of the type strain has been deposited in GenBank under accession number EU163502.

Emended description of *Streptococcus infantarius* subsp. *coli* Schlegel et al. (2000), corr. Beck, Frodl, and Funke 2008. *Streptococcus infantarius* subsp. *coli* (in.fan.ta'ri.us. L. adj. infantarius relating to infants, the source of the type strain; co'li. Gr. n. *colon*, colon; gen. n. *coli*, of colon).

The cells are gram-positive cocci that occur in pairs or short chains and are nonmotile, nonsporulating, and catalase negative. Colonies on blood agar are circular, 1 mm in diameter after 24 h incubation at 37°C, unpigmented, and alpha-hemolytic. Growth is enhanced in a 5% CO_2 atmosphere. Strains show homogeneous growth in buffer dextrose and in brain-heart infusion broths. Growth also occurs in MRS broth, without gas production. No exopolysaccharide production on 5% sucrose medium is observed. Strains are positive for Voges-Proskauer, leucine aminopeptidase, and alanyl-phenylalanyl-proline arylamidase tests. Arginine dihydrolase, alkaline phosphatase, and pyrrolidonyl-arylamidase tests are negative. Urea and hippurate are not hydrolyzed. Esculin hydrolysis is variable. Nearly all strains are α-galactosidase positive. They are negative for *N*-acetyl-β-glucosaminidase, β-galactosidase, β-glucuronidase, glycyl-tryptophan arylamidase, and β-mannosidase. All strains produce acid from lactose, maltose, sucrose, and starch. They do not produce acid from arabinose, arabitol, cyclodextrine, inulin, D-mannitol, melezitose, ribose, sorbitol, and D-tagatose. Variable results occur with glycogen, trehalose, melibiose, methyl-h-D-glucopyranoside, pullulan, and D-raffinose. The type strain for this subspecies is HDP 90246^T (= CCUG 47831^T = NCDO 964^T). The 16S rRNA gene sequence of the type strain has been deposited in GenBank under accession number EU163503.

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REFERENCES

1. **Clinical and Laboratory Standards Institute.** 2007. Performance standards for antimicrobial susceptibility testing; 17th informational supplement. Document M100-S17, CLSI, Wayne, PA.
2. **Corredoira, J. C., M. P. Alonso, J. F. García, E. Casariego, A. Coira, A. Rodriguez, J. Pita, C. Louzao, B. Pombo, M. J. López, and J. Varela.** 2005. Clinical characteristics and significance of *Streptococcus salivarius* bacteremia and *Streptococcus bovis* bacteremia: a prospective 16-year study. Eur. J. Clin. Microbiol. Infect. Dis. **24:**250–255.
3. **Facklam, R. R.** 1972. Recognition of group D streptococcal species of human origin by biochemical and physiological tests. Appl. Microbiol. **23:**1131–1139.
4. **Farrow, J. A. E., J. Kruze, B. A. Phillips, A. J. Bramley, and M. D. Collins.** 1984. Taxonomic studies on *Streptococcus bovis* and *Streptococcus equinus*: description of *Streptococcus alactolyticus* sp. nov. and *Streptococcus saccharolyticus* sp. nov. Syst. Appl. Microbiol. **5:**467–482.
5. **Funke, G., R. Frodl, and H. Sommer.** 2004. First comprehensively documented case of *Paracoccus yeei* infection in a human. J. Clin. Microbiol. **42:**3366–3368.
6. **Giannitsioti, E., C. Chirouze, A. Bouvet, I. Béguinot, F. Delahaye, J.-L. Mainardi, M. Celare, L. Mihaila-Amrouche, V. L. Moing, B. Hoen, et al.** 2007. Characteristics and regional variations of group D streptococcal endocarditis in France. Clin. Microbiol. Infect. **13:**770–776.
7. **Herrero, I. A., M. S. Rouse, K. E. Piper, S. A. Alyaseen, J. M. Steckelberg, and R. Patel.** 2002. Reevaluation of *Streptococcus bovis* endocarditis cases from 1975 to 1985 by 16S ribosomal DNA sequence analysis. J. Clin. Microbiol. **40:**3848–3850.
8. **Klein, R. S., R. A. Recco, M. T. Catalano, S. C. Edberg, J. I. Casey, and N. H. Steigbigel.** 1977. Association of *Streptococcus bovis* with carcinoma of the colon. N. Engl. J. Med. **297:**800–802.
9. **Kornbluth, A. A., J. B. Danzig, and L. H. Bernstein.** 1989. *Clostridium septicum* infection and associated malignancy. Report of 2 cases and review of the literature. Medicine (Baltimore) **68:**30–37.
10. **Moet, G. J., M. J. Dowzicky, and R. N. Jones.** 2007. Tigecycline (GAR-936) activity against *Streptococcus gallolyticus* (*bovis*) and viridans group streptococci. Diagn. Microbiol. Infect. Dis. **57:**333–336.
11. **Osawa, R., T. Fujisawa, and L. I. Sly.** 1995. *Streptococcus gallolyticus* sp. nov.; gallate degrading organisms formerly assigned to *Streptococcus bovis*. Syst. Appl. Microbiol. **18:**74–78.
12. **Poyart, C., G. Quesne, and P. Trieu-Cuot.** 2002. Taxonomic dissection of the *Streptococcus bovis* group by analysis of manganese-dependent superoxide dismutase gene (*sodA*) sequences: reclassification of '*Streptococcus infantarius* subsp. *coli*' as *Streptococcus lutetiensis* sp. nov. and of *Streptococcus bovis* biotype II.2 as *Streptococcus pasteurianus* sp. nov. Int. J. Syst. Evol. Microbiol. **52:**1247–1255.
13. **Ruoff, K. L., S. I. Miller, C. V. Garner, M. J. Ferraro, and S. B. Calderwood.** 1989. Bacteremia with *Streptococcus bovis* and *Streptococcus salivarius*: clinical correlates of more accurate identification of isolates. J. Clin. Microbiol. **27:**305–308.
14. **Schlegel, L., F. Grimont, E. Ageron, P. A. D. Grimont, and A. Bouvet.** 2003. Reappraisal of the taxonomy of the *Streptococcus bovis*/*Streptococcus equinus* complex and related species: description of *Streptococcus gallolyticus subsp. gallolyticus* subsp. nov., *S. gallolyticus* subsp. *macedonicus* subsp. nov. and *S. gallolyticus* subsp. *pasteurianus* subsp. nov. Int. J. Syst. Evol. Micribiol. **53:** 631–645.
15. **Schlegel, L., F. Grimont, M. D. Collins, B. Régnault, P. A. D. Grimont, and A. Bouvet.** 2000. *Streptococcus infantarius* sp. nov., *Streptococcus infantarius* subsp. *infantarius* subsp. nov. and *Streptococcus infantarius* subsp. *coli* subsp. nov., isolated from humans and food. Int. J. Syst. Evol. Microbiol. **50:**1425–1434.
16. **Spellerberg, B., and C. Brandt.** 2007. *Streptococcus*, p. 412–429. *In* P. R. Murray, E. J. Baron, J. H. Jorgensen, M. L. Landry, and M. A. Pfaller (ed.), Manual of clinical microbiology, 9th ed. ASM Press, Washington, DC.
17. **Thornsberry, C., C. N. Baker, and R. R. Facklam.** 1974. Antibiotic susceptibility of *Streptococcus bovis* and other group D streptococci causing endocarditis. Antimicrob. Agents Chemother. **5:**228–233.
18. **Tripodi, M.-F., R. Fortunato, R. Utili, M. Triassi, and R. Zarrilli.** 2005. Molecular epidemiology of *Streptococcus bovis* causing endocarditis and bacteraemia in Italian patients. Clin. Microbiol. Infect. **11:**814–819.