Mobile genetic elements provide evidence for a bovine origin of clonal complex 17 of Streptococcus agalactiae

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We sought an explanation for epidemiological changes in *Streptococcus agalactiae* infections, by investigating the link between ecological niches of the bacterium, by determining the prevalence of 11 mobile genetic elements. The prevalence of nine of these elements differed significantly according to the human or bovine origin of the isolate. Correlating this distribution with the phylogeny obtained by multilocus sequence analysis, we observed that human isolates harboring GBSi1, a clear marker of the bovine niche, clustered in clonal complex 17. Our results are thus consistent with the emergence of this virulent human clone from a bovine ancestor.

Epidemiological changes in the pattern of *Streptococcus agalactiae* infections over the last six decades, from predominantly bovine infections (13), to a leading cause of neonatal infections (25, 26) and, more recently, a cause of invasive disease in elderly adults (6), requires explanation. Genomic lineages grouping the most invasive isolates have been described by multilocus enzyme electrophoresis (17, 23), by pulsed-field gel electrophoresis (1, 24) and more recently by the analysis of the polymorphism in the allelic profiles of housekeeping genes (11), but are insufficient to account for the emergence of *S. agalactiae* from such diverse ecosystems. A link between the bovine and human niches has been proposed (8, 19), but remains a matter of debate. Indeed, it was recently suggested that human and bovine *S. agalactiae* isolates are largely unrelated (3) and represent distinct populations or subtypes (5, 27). Conversely, another study provided evidence to suggest that the major hyperinvasive ST-17 clone implicated in neonatal invasive diseases had arisen from bovine lineage ST-61 (2), whereas others suggested that the genome of the common ancestor was closer to that of human ST-17 strains than to that of the ST-61 strains of bovine origin (4). We hypothesized that mobile genetic elements (MGEs), such as insertion sequences (ISs) or group II introns, which drive bacterial evolution (12, 30), might provide insight into changes in the epidemiology of *S. agalactiae* infections.

We tested this hypothesis by analyzing a collection of 98 epidemiologically unrelated isolates, mostly collected in France (3 isolates from Germany and 1 from Malaysia) between 1966 and 2003: 63 human isolates from colonized individuals, adults with invasive diseases and neonates with meningitis (52 of which were previously typed by MLST and studied for the prevalence of IS*1548*, IS*861*, IS*1381*, ISSa4 and GBSi1) (10), and 35 bovine isolates obtained from cows with clinical evidence of udder infection.

The prevalence of 11 MGEs [one group II intron (GBSi1), and ten IS elements corresponding to six insertion sequences (IS*1548*, IS*861*, IS*1381*, ISSa4, ISSag1 and ISSag2), and four transposase genes corresponding to remnants of IS elements (ISSag9, *sag0448*, *sag1241*, and *sag0610*, relating to the identification of the ORFs in strains A909 and 2605V/R) (28, 29)], was evaluated by PCR. IS*1548*, IS*861*, IS*1381*, ISSa4, ISSag1, ISSag2 and GBSi1 were detected as previously described (10). Other transposase genes were detected with the primers listed in Table 1. The prevalence of all the MGEs except IS*861* and ISSag2 differed significantly as a function of the original reservoir (Fig. 1). This was particularly true for IS*1381*, IS*1548*, ISSag1 and ISSag9, which were mostly present in human isolates, and for GBSi1 and ISSa4, which were mostly present in bovine isolates (Fig. 1).

Multilocus sequence analysis, carried out with the standard multilocus sequence typing (MLST) scheme described by Jones *et al*. (11), resolved the 98 isolates into 47 STs. A phylogenetic tree was generated, based on the nucleotide sequences of the supergene obtained by concatenation of the seven loci for the 98 isolates, using MEGA software version 3.1 (www.megasoftware.net/) with the neighbor joining (NJ) method (14). Clonal complexes (CCs) (isolates sharing six or seven identical alleles) were defined by eBURST analysis (http://eburst.mlst.net/) (7). Seven main NJ phylogenetic divisions were identified, closely matching the seven main CCs identified by eBURST analysis (Fig. 2): subCC19 (n=21), subCC2 (n=11), CC10 (n=12), CC7 (n=6), CC17 (n=13), CC67 (n=15) and CC23 (n=10). Three of these CCs were “host-specific”: CC17 and subCC19 consisted almost exclusively of human isolates (100% and 95% of the isolates, respectively), whereas CC67 was almost exclusively composed of bovine isolates (93.8% of the isolates). Moreover, the NJ dendrogram shows that human

lineage CC17 arose from bovine lineage CC67 (Fig. 2), consistent with the assertions of Bisharat *et al*. (2).

A factorial analysis of correspondence (FAC) was performed on the whole data set, with the 11 MGEs as active variables and the three main clonal complexes (CC19, CC17 and CC67) and the bovine or human origins of the strains as illustrative variables. Computations were performed with SPAD.N software (Centre International de Statistiques et Informatique Appliquées, St Mandé, France), as previously described (22). The projections of the active and illustrative variables and of the strains on the F1/F2 plane accounting for 40.97 % of the total variance (Fig. 3), separated two groups of variables and of strains. GBSi1 and ISSa4 (and to a lesser degree *sag0448* and *sag1241*), bovine origin, CC17 and CC67 and 94.3% of bovine isolates were projected onto the negative values of F1. IS*1548*, IS*1381*, ISSag9 and ISSag1 (and to a lesser degree *sag0610*), human origin, CC19 and 79.4% of human isolates were projected onto the positive values of F1. These findings confirm those of Luan *et al.* in that the two major human *S. agalactiae* lineages are each marked by one MGE: subCC19 (defined as CC19 by Luan *et al*.) (16), is characterized by IS*1548*, whereas CC17 is characterized by GBSi1. All but one of the human isolates projected with the bovine isolates and variable CC67 onto the negative values of F1 belonged to CC17, highlighting the stronger linkage of CC17 with the bovine lineage CC67 than with any of the other human lineages (Fig. 3). Moreover, our data also suggest that the presence of GBSi1 in the bovine reservoir predates its presence in the human reservoir because (1) all our bovine isolates harbored GBSi1 whereas this element was present in less than one third of human isolates (almost all of which belonged to CC17) and (2) the bovine isolates had higher copy numbers and more complex patterns for GBSi1 than the human isolates (data not shown), consistent with an increase in IS pattern complexity over time (18).

MGEs therefore provide evidence for a bovine ancestor of the human clonal lineage CC17, all the isolates of which harbor GBSi1. This statement is consistent with the proposed evolutionary scheme of MGE acquisition, in which GBSi1 is thought to have been acquired recently by human isolates (10).

The particular distribution of MGEs among bovine and human isolates (Fig. 3) is also consistent with a physical barrier reducing the exchange of genetic material between the bovine and human reservoirs. The human isolate H14, which occupies a prominent position on the NJ dendrogram, between the bovine CC67 and the human CC17 (Fig. 2), displayed two particular features. First, the copy number of GBSi1 in this isolate was much higher than that in other isolates (human isolates had a median of 3 GBSi1, whereas H14 harbored 12 copies — data not shown). Parkhill *et al.* suggested that IS expansion occurs during evolutionary bottlenecks (20, 21). They argued that in *Yersinia,* IS expansion coincided with a change in the ecological niche of the species, from commensal organism of the gut to systemic pathogen (21). Second, isolate H14 harbored both IS*1548* and GBSi1. In our study, as in previous studies (9, 15), very few isolates harbored both GBSi1 and IS*1548* (see Fig. 2). Granlund *et al*. explained this mutually exclusive distribution by the presence of a closed insertion site for the two MGEs on the bacterial chromosome (9), but this distribution could also be explained by IS*1548* and GBSi1 originating from two different ecological niches. Thus, human clone CC17 seems to have evolved from the bovine lineage containing GBSi1, and has retained the GBSi1 genetic marker from its bovine donor.

In conclusion, our results provide at least a partial explanation of the emergence of *S. agalactiae* as a major cause of neonatal meningitis, despite its original identification as the causal agent of bovine mastitis. Assuming that CC17 evolved from a bovine ancestor, as shown both by MLST analysis and by the presence of the bovine

130 marker GBSi1, the isolates of this clonal complex may have genetic features different
131 from those of other *S. agalactiae* isolates that have adapted to their human hosts over a
132 longer period of time. These features probably concern genes not present in the core
133 genome, as recently defined by Tettelin *et al*., but that have been acquired by horizontal
134 exchange due to the environmental gene pool available in other ecosystems (28).
135 Indeed, we recently reported a link between prophage DNA fragments and *S. agalactiae*
136 isolates from neonatal cases of meningitis, suggesting the presence of additional genetic
137 material in these strains (10).
138
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271 TABLE 1. Primers used for the amplification of transposase genes from the
272 *S. agalactiae* isolates.
273

Target	Genbank accession no.[a]	Forward primer (5'-3')	Reverse primer (5'-3')	Tm (°C)	Amplicon (bp)
ISSag9	NC_004116	gaccgtaatgacatccaatctgg	ggcttgaaatggacacggtt	50	600
sag0448	NC_004116	ccacagaattacttaacttcctagc	cgaccaaccttattgaatcact	50	994
sag1241 (*orfA*)	NC_004116	ctcttgttaacctttatcaaactgg	cctaaaaaaagcaagtgcca	50	218
sag0610	NC_004116	tactccacattagctaaagaacagg	ggcctgatttaaccgagatt	50	394

274
275 [a] NC_004116 refers to strain 2603 V/R (29)

FIG. 1. Prevalence of the mobile genetic elements within the human (filled bars) and the bovine (open bars) *S. agalactiae* isolates. Significant differences (without correction for bias due to multiple comparisons) are indicated by one asterisk for p values below 0.05 and two asterisks for p values below 0.001.

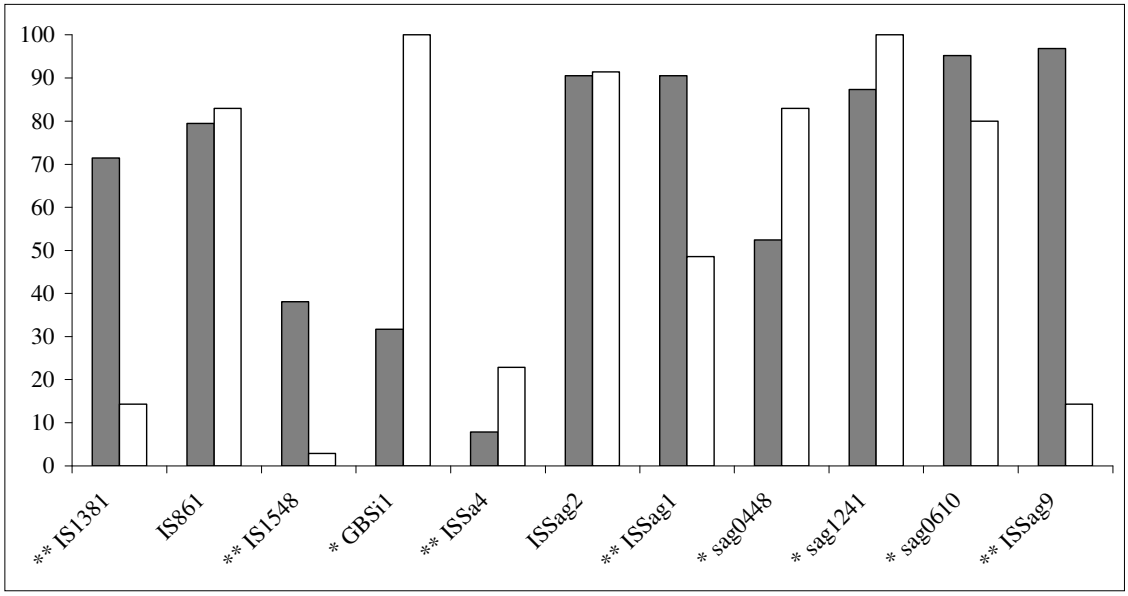


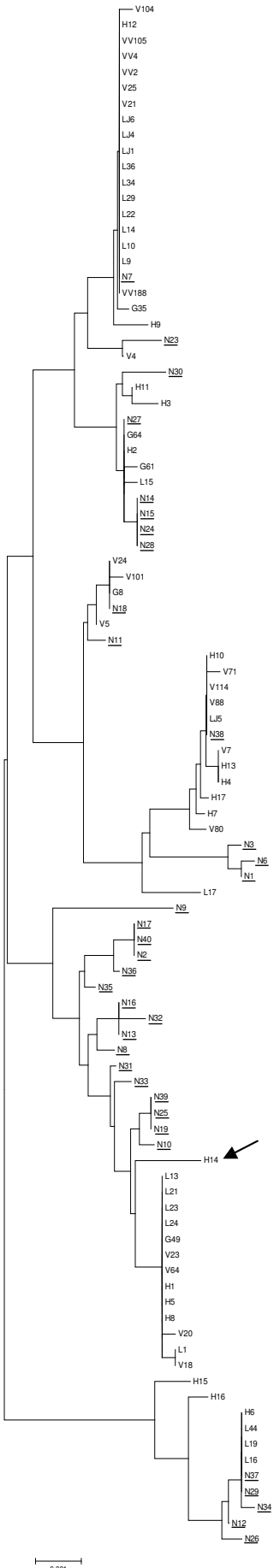
FIG. 2. Relatedness of the 98 isolates of *S. agalactiae* analyzed by the NJ method, using MEGA v3.1 software and the Kimura 2-parameter mutation model of genetic distance. The nucleotide differences between the supergene sequences obtained in multilocus sequence analysis, as described by Jones *et al.* (11), were used to infer phylogenetic relationships between isolates. We used eBURST v3 software to group the 98 isolates into clonal complexes (CC). For each isolate, sequence type (ST), ß-hemolysis, serotype and presence/absence of the 11 mobile genetic elements (MGEs) are indicated. Clonal complexes are also indicated on the right of the figure (several isolates were not attributed a CC number by the Start software and are considered "singletons").

The particular position of isolate H14 is indicated by a black arrowhead.

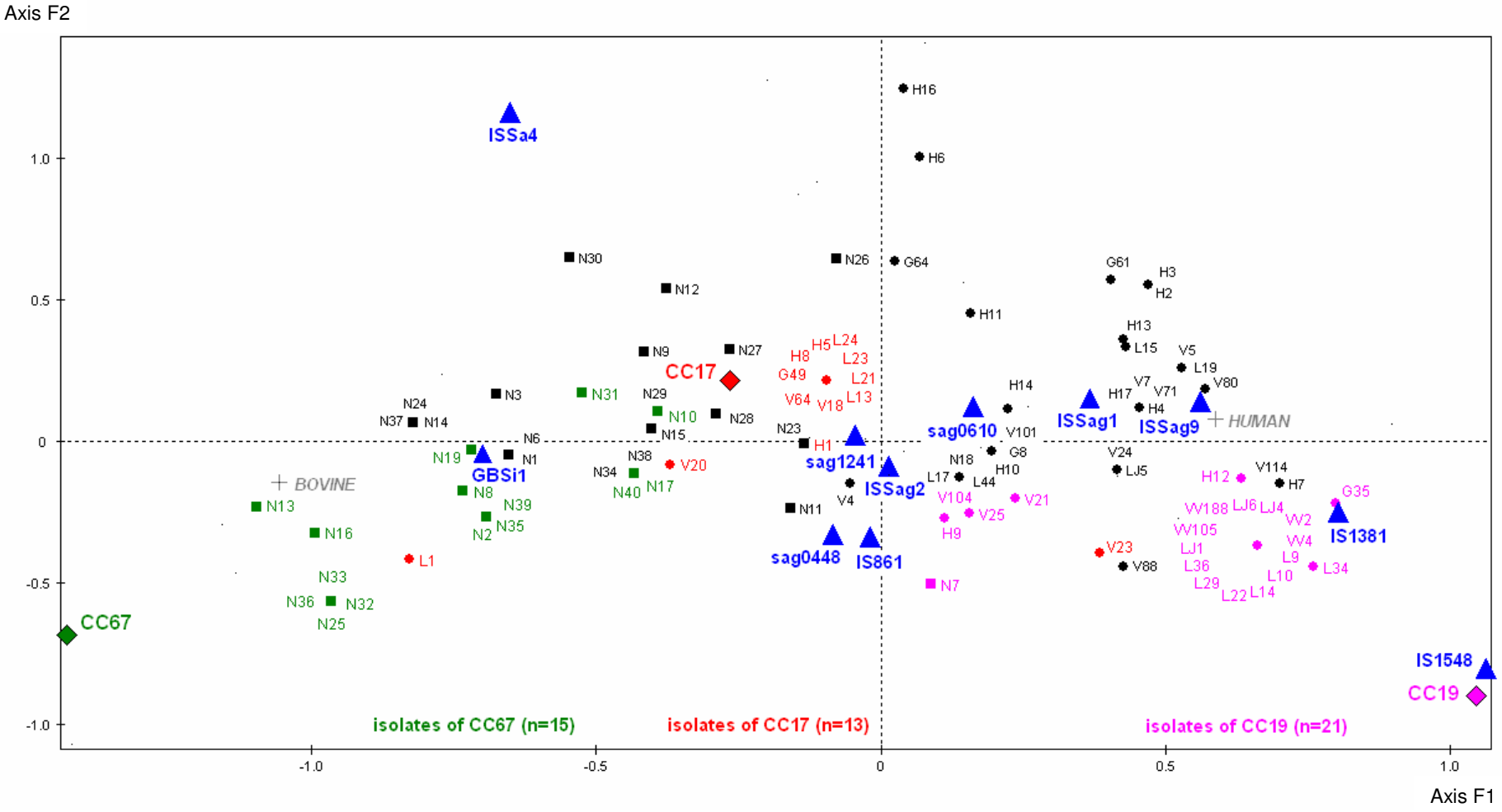
The human isolates are named L or LJ (meningocerebral fluid), H (adult), V or VV (vaginal carriage) or G (gastric fluid); the bovine isolates are named N (bovine isolates are underlined).

NT: non typable

FIG. 3. Factorial analysis of correspondence of 98 *S. agalactiae* isolates based on the mobile genetic element (MGE) data. Eleven active variables [the MGEs (▲) IS*1381*, IS*861*, IS*1548*, GBSi1, ISSa4, ISSag2, ISSag1, *sag0448*, *sag1241*, *sag0610* and ISSag9], and five illustrative variables [the main clonal complexes CC19 (♦), CC17 (♦) and CC67 (♦), and the human (●) and bovine (■) origins of the isolates], and the 98 isolates are projected onto the F1/F2 plane. This plane, obtained by computation, is defined by the two principal axes of the analysis; the first axis, F1, explains most of the variance, and the second axis, F2 (orthogonal to F1), explains most of the variance not explained by F1. Together, F1 and F2 accounted for 40.97% of the total variance. Isolates of CC17 (n=13) are shown in red, isolates of CC67 (n=15) in green, and isolates of subCC19 (n=21) in pink.



Isolate	Sequence Type	β-haemolysis	Serotype	MGEs											
				GBSi1	IS*1381*	IS*861*	IS*1548*	ISSa4	ISSag2	ISSag1	*sag0448*	*sag1241*	*sag0610*	ISSag9	
V104	ST 28	+	II	+	+	+	-	-	+	+	+	+	+	+	subCC19
H12	ST 19	+	III	-	+	+	+	-	-	+	+	+	+	+	
VV105	ST 19	+	III	-	+	+	+	-	+	+	+	+	+	+	
VV4	ST 19	+	III	-	+	+	+	-	+	+	+	+	+	+	
VV2	ST 19	+	III	-	+	+	+	-	+	+	+	+	+	+	
V25	ST 19	+	NT	-	+	+	-	-	+	-	+	+	+	+	
V21	ST 19	+	II	+	+	+	-	-	+	+	+	-	+	+	
LJ6	ST 19	+	III	-	+	+	+	-	+	+	+	+	+	+	
LJ4	ST 19	+	III	-	+	+	+	-	+	+	+	+	+	+	
LJ1	ST 19	+	III	-	+	+	+	-	+	+	+	+	+	+	
L36	ST 19	+	III	-	+	+	+	-	+	+	+	+	+	+	
L34	ST 19	+	III	-	+	+	+	-	+	+	+	-	+	+	
L29	ST 19	+	III	-	+	+	+	-	+	+	+	+	+	+	
L22	ST 19	+	III	-	+	+	+	-	+	+	+	+	+	+	
L14	ST 19	+	III	-	+	+	+	-	+	+	+	+	+	+	
L10	ST 19	+	III	-	+	+	+	-	+	+	+	+	+	+	
L9	ST 19	+	III	-	+	+	+	-	+	+	+	+	+	+	
N7	ST 19	+	III	+	+	+	+	-	+	+	+	+	+	-	
VV188	ST 19	+	III	-	+	+	+	-	+	+	+	+	+	+	
G35	ST 197	+	NT	-	+	+	+	-	+	+	-	-	+	+	
H9	ST 131	+	V	+	+	+	+	-	+	+	+	+	+	+	
N23	ST 226	+	II	+	-	+	-	-	+	+	+	+	+	+	subCC2
V4	ST 366	+	Ia	-	-	-	+	-	+	-	+	-	+	-	
N30	ST 261	+	IV	+	-	-	-	+	+	+	+	+	+	-	
H11	ST 1	+	NT	-	-	-	-	-	+	+	+	+	+	+	
H3	ST 186	+	NT	-	+	-	-	-	+	+	-	+	+	+	
N27	ST 2	-	II	+	-	+	-	+	+	+	+	+	+	+	
G64	ST 2	+	II	+	+	-	-	+	+	+	+	+	+	+	
H2	ST 2	+	NT	-	+	-	-	-	+	+	-	+	+	+	
G61	ST 202	+	V	-	+	-	-	-	-	+	+	+	+	+	
L15	ST 196	+	IV	-	+	-	-	-	+	+	+	+	+	+	
N15	ST 250	+	NT	+	+	-	-	-	+	-	+	+	+	-	
N14	ST 250	+	NT	+	-	+	-	+	+	-	+	+	+	-	
N24	ST 357	+	NT	+	-	+	-	+	+	-	+	+	+	-	
N28	ST 250	+	NT	+	+	+	-	+	+	+	+	+	+	-	
V24	ST 7	+	IV	-	+	+	-	-	+	+	+	+	+	+	CC7
V101	ST 41	+	V	-	+	+	-	-	+	-	-	+	+	+	
G8	ST 7	+	Ia	-	+	+	-	-	+	-	-	+	+	+	
N18	ST 7	+	Ia	+	+	+	-	-	+	+	+	+	+	+	
V5	ST 6	+	Ib	-	+	-	-	-	+	+	+	-	+	+	
N11	ST 354	+	Ib	+	+	+	-	-	+	+	+	+	+	-	
H10	ST 10	+	Ib	-	+	+	-	-	+	-	-	+	+	+	CC10
V71	ST 8	+	Ib	-	+	+	-	-	+	+	-	+	+	+	
V114	ST 10	+	Ib	-	+	+	+	-	+	+	-	+	+	+	
V88	ST 10	+	II	-	+	+	+	-	+	+	-	+	-	+	
LJ5	ST 10	+	Ib	-	+	+	-	-	+	+	+	+	+	+	
N38	ST 10	+	Ib	+	-	+	-	-	+	+	+	+	+	-	
V7	ST 12	+	Ib	-	+	+	-	-	+	+	-	+	+	+	
H13	ST 12	+	Ib	-	+	+	-	-	-	+	-	+	+	+	
H4	ST 12	+	Ib	-	+	+	-	-	+	+	-	+	+	+	
H17	ST 200	+	Ib	-	+	+	-	-	+	+	-	+	+	+	
H7	ST 10	+	II	-	+	+	+	-	+	+	-	+	+	+	
V80	ST 12	+	II	-	+	+	+	+	+	+	-	+	+	+	
N3	ST 352	-	NT	+	-	-	-	-	+	-	+	+	+	-	**CC67**
N6	ST 353	-	NT	+	-	+	-	-	+	-	-	+	+	-	
N1	ST 350	+	NT	+	-	+	-	-	+	-	-	+	+	-	
L17	ST 195	+	III	+	+	+	-	-	+	+	+	+	+	+	
N9	ST 26	+	V	+	-	-	-	-	+	+	+	+	+	-	
N17	ST 351	-	II	+	-	+	-	-	+	+	+	+	+	-	
N40	ST 351	-	NT	+	-	+	-	-	+	+	+	+	+	-	
N2	ST 351	-	II	+	-	+	-	-	+	-	+	+	+	-	
N36	ST 365	+	II	+	-	+	-	-	+	-	+	+	-	-	
N35	ST 364	-	NT	+	-	+	-	-	+	-	+	+	+	-	
N16	ST 67	+	NT	+	-	+	-	-	-	-	+	+	-	-	
N32	ST 85	+	NT	+	-	+	-	-	+	-	+	+	-	-	
N13	ST 67	+	NT	+	-	+	-	+	+	-	+	+	-	-	
N8	ST 64	-	II	+	-	+	-	-	-	+	+	+	-	-	
N31	ST 361	-	III	+	-	+	-	+	+	-	+	+	+	+	
N33	ST 362	-	III	+	-	+	-	-	+	-	+	+	-	-	
N39	ST 61	+	NT	+	-	+	-	-	+	-	+	+	+	-	
N25	ST 61	+	III	+	-	+	-	-	+	-	+	+	-	-	
N19	ST 61	+	NT	+	-	+	-	-	-	-	+	+	+	-	
N10	ST 63	+	II	+	-	+	-	-	+	+	-	+	+	-	
H14	ST 22	+	II	+	+	+	+	-	-	+	-	-	+	+	
L13	ST 17	+	III	+	-	+	-	-	+	+	-	+	+	+	**CC17**
L21	ST 17	+	III	+	-	+	-	-	+	+	-	+	+	+	
L23	ST 17	+	III	+	-	+	-	-	+	+	-	+	+	+	
L24	ST 17	+	III	+	-	+	-	-	+	+	-	+	+	+	
G49	ST 17	+	III	+	-	+	-	-	+	+	-	+	+	+	
V23	ST 17	+	NT	+	+	+	+	-	+	+	+	+	+	+	
V64	ST 17	+	III	+	-	+	-	-	+	+	-	+	+	+	
H1	ST 17	+	III	+	-	+	-	-	+	+	+	+	+	+	
H5	ST 17	+	III	+	-	+	-	-	+	+	-	+	+	+	
H8	ST 17	+	III	+	-	+	-	-	+	+	-	+	+	+	
V20	ST 367	+	III	+	-	+	-	-	+	+	-	+	-	+	
L1	ST 201	+	III	+	-	+	-	-	+	-	-	-	-	-	
V18	ST 201	+	III	+	-	+	-	-	+	+	-	+	+	+	
H15	ST 198	+	III	-	-	-	-	+	-	+	-	+	+	+	CC23
H16	ST 199	+	III	-	-	-	-	+	-	+	-	+	+	+	
H6	ST 23	+	III	-	-	-	-	+	+	+	-	+	+	+	
L44	ST 23	+	Ia	+	+	+	-	-	+	+	+	+	+	+	
L19	ST 23	+	Ia	-	+	-	-	-	+	+	+	-	+	+	
L16	ST 23	+	III	-	-	-	-	-	+	+	+	+	+	+	
N37	ST 23	+	III	+	-	+	-	+	+	-	+	+	+	-	
N29	ST 23	+	III	+	-	+	-	-	+	+	-	+	+	-	
N34	ST 363	+	III	+	-	+	-	-	+	+	+	+	+	-	
N12	ST 355	+	III	+	-	-	-	-	+	+	-	+	+	-	
N26	ST 358	+	III	+	-	-	-	-	+	+	-	+	+	+	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-1.0
-0.5
0
0.5
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CC17
CC67
CC19
GBSi1
sag1241
sag0610
sag0448
ISSag2
IS861
ISSag1
ISSag9
IS1381
IS1548
BOVINE
HUMAN
isolates of CC67 (n=15)
isolates of CC17 (n=13)
isolates of CC19 (n=21)