High similarity and high frequency of virulence genes among *Salmonella* Dublin strains isolated over a 33-year period in Brazil

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Abstract

Salmonella Dublin is a strongly adapted serovar that causes enteritis and/or systemic disease with high rates of mortality in cattle and occasionally infects humans. Despite the importance of this serovar, there is a lack of studies in Brazil. The aim of this study was to characterize the genetic diversity of 112 *S.* Dublin strains isolated from humans and animals in Brazil by CRISPR and CRISPR-MVLST and the relatedness among strains by MLST. In addition, the frequency of some important virulence genes was verified. The strains studied belonged to nine different sequence types, being all of them single- or double-locus variants of the ST10. CRISPR discriminated the strains into 69 subtypes with a similarity ≥ 84.4% and CRISPR-MVLST into 72 subtypes with a similarity ≥ 84.7%. The virulence genes *ratB*, *lpfA*, *mgtC*, *avrA*, *sopB*, *sopE2*, *sifA*, *sseA*, *ssrA*, *csgA*, *fliC*, and *sinH* were found in all the strains studied, while *spvB*, *spvC*, *sodCl*, *rpoS*, *sipA*, *sipD*, *invA*, and *hilA* were detected in ≥ 93.7% of the strains. In conclusion, the high similarity among the strains reinforces the clonal nature of the strains of this serovar that may have descended from a common ancestor that little differed over 33 years in Brazil. CRISPR and CRISPR-MVLST showed to be good alternatives to type *S.* Dublin strains. MLST suggested that *S.* Dublin strains from Brazil were phylogenetically related to strains from other parts of the globe. Moreover, the high frequency of virulence genes among the strains studied reinforces the capacity of *S.* Dublin to cause invasive diseases.

Keywords *Salmonella* Dublin · MLST · CRISPR · CRISPR-MVLST · Virulence genes

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Introduction

Salmonellosis caused by non-typhoidal serovars is among the most common foodborne illnesses worldwide, accounting for 93.8 million cases of gastroenteritis and 155,000 deaths annually [1].

Salmonella enterica serovar Dublin (*S.* Dublin) is strongly adapted to cattle, responsible for causing enteritis and/or systemic disease with high rates of mortality. This fact becomes even more concerning due to the negative economic impact for many beef-producing countries, since *S.* Dublin infections in cattle may result in reduced milk production, abortion in pregnant cows, and eventually in deaths [2–4].

Occasionally, *S.* Dublin can also be isolated from serious and even fatal infections in humans, especially in patients with underlying immunosuppression conditions, and usually causing a serious disease that can even be indistinguishable from typhoid fever [2–4].

Similarly to most *Salmonella* serovars, the pathogenesis of *S.* Dublin strains is achieved, among others, by proteins coded by chromosomal genes responsible for multiple cellular functions as adhesion, acid and serum resistance, invasion of host cells, and survival within phagocytic cells. Such genes are mostly located in the *Salmonella* pathogenicity islands (SPIs), in special the most studied ones, SPI-1 and SPI-2. In addition, virulence plasmids such as the *pSDL*, that carries the *spv* operon, also play important roles in survival and growth of *Salmonella* Dublin into macrophages [5–9].

Some methodologies such as pulsed-field gel electrophoresis (PFGE), multiple-locus variable-number of tandem repeats analysis (MLVA), enterobacterial repetitive intergenic consensus PCR (ERIC-PCR), multilocus enzyme electrophoresis (MLEE), multilocus sequence typing (MLST), and clustered regularly interspaced short palindromic repeats (CRISPR) have been successfully used to subtype strains of many *Salmonella* serovars, including *S.* Dublin [9–13]. Although the great applicability, some methodologies, such as MLST and CRISPR, require excessive laboratory work and high costs that restricts the performing of these methodologies to small and representative sets of strains [14–17]. However, the evolution and costs reductions in whole-genome sequencing (WGS) have been providing a wider access to sequence larger sets of bacterial strains and consequently have been allowing different molecular analysis to be performed in a faster and easier way than the traditional typing [18, 19].

MLST is a method based on the analysis of a particular set of housekeeping genes for each bacterial species. The analysis of the alleles of seven housekeeping genes can be submitted to an online public database (https://enterobase.warwick.ac.uk/) that assigns the strains according to the specific alleles to a specific sequence type (ST), which allows the comparison of strains isolated in different parts of the globe [15]. This methodology has successfully contributed to the understanding of the epidemiology, evolution, and genotypic diversity of many *Salmonella* serovars and it has even been proposed as an alternative for traditional identification by serotyping [15, 19, 20].

CRISPR are short and highly conserved sequences of DNA direct repeats, which range from 21 to 48 base pairs (bp), usually specific for a determined CRISPR locus. These sequences are regularly interspaced by variable DNA sequences of constant and similar length, usually 20–58 bp, called spacers, which vary according to the species of the microorganisms or the CRISPR locus [21, 22]. Among *Salmonella* serovars, two non-coding CRISPR loci were identified in their genomes, and the analysis of the different spacers contained in their respective CRISPRs loci has been successfully used to subtype these serovars [18, 23–25].

Aiming to increase the discriminatory power of CRISPR technique, Liu et al. (2011) proposed the association of the two *Salmonella* CRISPR loci with the virulence genes *fimH*, responsible for bacterial binding to structures in the cell-host membrane, and *sseL*, responsible for inducing inflammation and killing macrophages [28]. This association originated the method known as CRISPR-multi-locus virulence sequence typing (CRISPR-MVLST) [26–28]. This methodology has also been successfully used to subtype serovars as Enteritidis, Newport, and Typhimurium [18, 23–25].

Few information is available about the molecular epidemiology of *S.* Dublin strains isolated worldwide, and most of the studies did not study sets of strains exclusively of this serovar, interfering on the understanding of the specific characteristics and traits of *S.* Dublin [9–12, 29]. Specifically in Brazil, only five studies molecularly typed strains of this serovar, among which, only two analyzed a large set exclusive of *S.* Dublin strains, making it difficult to evaluate the diversity of strains of this serovar circulating in this country [13, 30–33]. Furthermore, MLST, CRISPR, and CRISPR-MVLST have never been used for typing strains of this serovar in Brazil, according to the published literature.

Therefore, the aim of this study was to genotype *S.* Dublin strains isolated from humans and animals in Brazil between 1983 and 2016 by MLST and CRISPR and its variation CRISPR-MVLST. Moreover, the ability of CRISPR-based methodologies in subtyping *S.* Dublin strains was analyzed in addition to the pathogenic potential of these strains that was determined by searching for the frequency of 20 genes related to *Salmonella* virulence.

Material and methods

Bacterial strains

A total of 112 *Salmonella* Dublin strains isolated in Brazil from humans (82) between 1983 and 2016, and animals (30) between 1992 and 2015 were studied. These strains were previously described in Vilela et al. 2018 [13] and are representative isolates of the years, states, material, and source of isolation of the collections of two *Salmonella* reference laboratories in Brazil, the Adolfo Lutz Institute of São Paulo (IAL-SP), and Oswaldo Cruz Foundation of Rio de Janeiro (FIOCRUZ-RJ). Supporting Information Table S1 presents the year, source, and states of isolation of the 112 *S.* Dublin strains studied.

MLST

MLST was performed in silico for all the *S.* Dublin studied using the 112 whole-genome assembled sequences, previously obtained and described in Campioni et al. (2018) [34], following the Achtman scheme available at the Enterobase database (http://enterobase.warwick.ac.uk/species/senterica/

allele_st_search) using the allele identification of seven specific housekeeping genes for *S. enterica* (*aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA*). Allele identification was performed by uploading the assembled sequences of *S.* Dublin strains studied in the MLST web-based tool available in the Center for Genomic Epidemiology website (https://cge.cbs.dtu.dk/services/MLST/). The minimum spanning tree generated with STs and eBURST groups was generated with the software eBURSTv3.

CRISPR and *fimH* and *sseL* virulence genes analyses

The CRISPRs loci and the virulence genes *fimH* and *sseL* analysis were also performed for all the *S.* Dublin studied using the 112 whole-genome assembled sequences.

Analysis of CRISPR1 and CRISPR2 was performed, uploading the assembled sequences in the CRISPRFinder tool (available at http://crispr.i2bc.paris-saclay.fr/). This tool automatically analyzes the CRISPRs, as well as the length and location per contig of the direct repeats and spacers in each genome. To perform the analysis, only spacers were considered, as reported in previous studies [23–25, 28]. These spacers were manually listed in each of the *S.* Dublin strains studied, and a binary matrix with the presence or absence of every spacer in CRISPR1 and CRISPR2 was generated using Microsoft Excel.

The analysis of the sequences of the virulence genes *fimH* and *sseL* was performed using Basic Local Alignment Search Tool (BLAST) (available at blast.ncbi.nlm.nih.gov/Blast.cgi) by uploading the assembled sequences of all *S.* Dublin strains studied and aligning with sequences of the *fimH* and *sseL* genes, of 1005 bp and 954 bp, respectively, downloaded from GenBank (available at ncbi.nlm.nih.gov/genbank/) as standard (accession numbers KF465864 and KJ095841 for the genes *sseL* and *fimH*, respectively). The sequences were analyzed using ChromasPro 2.33 (Technelysium Pty. Ltd.).

CRISPR-MVLST sequence types (CM-ST) were assigned based on the combination of CRISPR1 and CRISPR2 profiles and *fimH* and *sseL* alleles for all strains studied. A similarity dendrogram was generated based on the CRISPRs binary matrices and another one was generated based on CRISPRs binary matrices in addition to the allele types of *fimH* and *sseL* genes. The software BioNumerics 7.6 (Applied Maths) with the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm was used to build both dendrograms. The discriminatory power of both CRISPR and CRISPR-MVLST was assessed by Simpson's diversity index, as described by Hunter and Gaston (1988) [35].

Virulence gene detection

All the *S.* Dublin strains studied were tested for the presence of 21 virulence genes using the *MyDbFinder* tool, a web-based

tool available in the Center for Genomic Epidemiology website (https://cge.cbs.dtu.dk/services/MyDbFinder/). Briefly, specific sequences of genes *ratB*, *sodCl*, *lpfA*, *rpoS*, *mgtC*, *sipA*, *sipD*, *invA*, *avrA*, *hilA*, *sopB*, *sopE2*, *sifA*, *sseA*, *ssrA*, *spvB*, *spvC*, *csgA*, *fljB*, *fliC*, and *sinH* were downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and combined in a single *fasta* format file to create a personalized database. This database was uploaded to *MyDbFinder* tool to perform the alignment between the assembled sequences of the *S.* Dublin strains studied with the sequences of the searched virulence genes and detecting if they were present or not among the strains studied. The parameters used were 90% of minimum identity and 60% of minimum length. The 21 virulence genes were searched; its respective functions and accession numbers are presented in Table 1.

Results

MLST

The STs detected, eBURST group, allelic profile of the housekeeping genes analyzed, and the percentage of detection among the strains studied are presented in Table 2. The minimum spanning tree generated with the STs detected is presented in Fig. 1. Among the *S.* Dublin strains studied, nine STs were detected and belonged to the same eBURST group eBG53 (Table 2). The most prevalent ST among the strains studied was ST10. The STs ST3734, ST4030, ST4097, ST4100, ST4232, and ST4574 were single-locus variants of the ST10, while ST4098 and ST4101 were double-locus variants of ST10 (Fig. 1, Table 2). Moreover, STs ST4097, ST4098, ST4100, ST4101, ST4232, and ST4574 were detected for the first time in the *S.* Dublin global database.

CRISPR and CRISPR-MVLST

Among the 112 *S.* Dublin strains studied, 59 CRISPR1, 12 CRISPR2, 2 *fimH*, and 4 *sseL* alleles were identified. When combined, these alleles generated 72 CM-STs (Table 3). CRISPR1 alleles ranged from one to nine spacers in size, while CRISPR2 alleles ranged from two to four spacers in size. In our analysis, we found 70 and 16 different spacers in CRISPR1 and CRISPR2 loci, respectively. The strain SD 721 presented a duplication in one of CRISPR1 spacers and was also included in the analysis. The complete absence of spacers was found in a single strain in CRISPR1 analysis and in 33 strains in CRISPR2 analysis.

The similarity dendrogram generated with the binary matrix of CRISPR1 and CRISPR2 spacers grouped all the 112 *S.* Dublin strains in a single cluster (> 80% of similarity) presenting 69 CRISPR-types with a similarity ≥ 84.4% among the strains (Fig. 2). The association of the binary matrix of

Table 1 Virulence genes searched in the 112 *S.* Dublin strains studied and its respective functions and accession numbers (GenBank)

Virulence gene	Function	Accession number
ratB	Putative outer membrane protein	NP_461449
sodCl	Gifsy-2 prophage: superoxide dismutase precursor (Cu-Zn)	NP_460019
lpfA	Long polar fimbria protein LpfA	NP_462541
rpoS	Sigma S (sigma 38) factor of RNA polymerase, the major sigma factor during stationary phase	NP_461845
mgtC	Mg^{2+} transport protein	NP_462663
sipA	Type III secretion system effector SipA, actin polymerizing activity	NP_461803
sipD	Type III secretion system hydrophilic translocator, needle tip protein SipD	NP_461804
invA	Type III secretion system major export apparatus protein InvA	NP_461817
avrA	Putative inner membrane protein	NP_461786
hilA	Invasion protein transcriptional activator	NP_461797
sopB	Invasion gene D protein	NP_460064
sopE2	Type III secretion protein SopE2	NP_460811
sifA	Replication in macrophages; SIFA protein	NP_460194
sseA	Chaperone for *sseB* and *sseD*	NP_460362
ssrA	Hybrid sensor histidine kinase/response regulator	NP_460357
spvB	Type III secretion system effector SpvB, ADP-ribosylation activity	NP_490529
spvC	Type III secretion system effector SpvC, phosphothreonine lyase	NP_490528
csgA	Curlin major subunit CsgA	NP_460115
fljB	Phase 2 flagellin; flagellar synthesis	NP_461698
fliC	Phase 1 flagellin; Filament structural protein	NP_460912
sinH	Intimin-like protein	NP_461452

CRISPR1 and CRISPR2 spacers with the respective *fimH* and *sseL* gene loci also grouped all the 112 *S.* Dublin strains into a single cluster presenting 72 CRISPR-MVLST-types with a similarity ≥ 84.7% among the strains studied (Fig. 3). The discriminatory index (DI) for CRISPR and CRISPR-MVLST were 0.976 and 0.980, respectively.

Detection of virulence genes

The frequency of 21 virulence genes showed that all the *S.* Dublin strains studied carried the genes *ratB*, *lpfA*, *mgtC*, *avrA*, *sopB*, *sopE2*, *sifA*, *sseA*, *ssrA*, *csgA*, *fliC*, and *sinH*. Moreover, *spvB* gene was present in 105 strains (93.7%), *spvC* in 106 strains (94.6%), *sodCl* in 108 strains 96.5%), *rpoS* in 109 strains (97.3%), *sipA* in 110 strains (98.2%), *sipD* in 110 strains (98.2%), *invA* in 110 strains (98.2%), and *hilA* was present in 110 strains (98.2%). On the other hand, *fljB* gene was not detected in any of the strains studied.

Discussion

Salmonella Dublin is a serovar strongly adapted to bovine hosts, but can be sporadically isolated from human clinical cases [1, 2]. Different molecular typing techniques have been used for epidemiological studies of *S.* Dublin strains worldwide [9–13]. The advancement in whole-genome sequencing allowed the sequencing of large sets of strains and the characterization by classic or newly developed methodologies, such as MLST and CRISPR [18, 19]. To our knowledge, no studies have been conducted to characterize the genotypic diversity in large sets composed exclusively of *S.* Dublin strains isolated in Brazil by MLST and/or CRISPR. In the present study, we used MLST, CRISPR, and CRISPR-MVLST to type 112 *S.* Dublin strains isolated from humans and animals between 1983 and 2016 in Brazil. In addition, we characterized the virulence potential of these strains searching for the frequency of 20 *S. enterica* virulence genes.

Table 2 Sequence type (ST), eBURST group (eBG), number of strains, year of isolation, and allelic profile of 112 *S.* Dublin strains studied

STs	eBG	Number of strains (%)	Year of isolation	*aroC*	*dnaN*	*hemD*	*hisD*	*purE*	*sucA*	*thrA*
10	53	68 (60.7)	1985–2016	5	2	3	6	5	5	10
3734	53	28 (25.0)	1984–2013	5	2	3	6	5	671	10
4030	53	9 (8.0)	1983–2005	5	2	612	6	5	5	10
4097	53	1 (0.9)	2007	5	2	3	6	748	5	10
4098	53	1 (0.9)	2005	5	2	612	6	749	5	10
4100	53	2 (1.8)	1991	5	2	3	6	5	713	10
4101	53	1 (0.9)	1998	5	2	3	6	750	671	10
4232	53	1 (0.9)	1988	5	2	3	6	636	5	10
4574	53	1 (0.9)	1990	5	2	3	6	800	5	10

Fig. 1 Minimum spanning tree generated with the software eBURSTv3 for the 112 *S.* Dublin strains studied and the other strains of this serovar available in the Enterobase database. Each ST is represented by a dot. The pink numbers above black dots represent the STs detected in this study. The blue central dot represents the predicted primary founder ST of the clonal complex 53 (CC53), ST10. The diameter of each dot indicates the prevalence of the STs in the input data that generated the graphic. Black numbers above back dots represent other *S.* Dublin STs presented in the database

Due to laborious work and high costs to perform traditional MLST for large sets of strains, most studies only used this methodology to type small sets of representative strains [14–17]. However, WGS has been providing a faster and easier alternative to type in silico a large number of strains, contributing for a better characterization of many *Salmonella* serovars [18, 19].

In the present study, MLST revealed the presence of nine different STs among the strains studied (Table 2). The most prevalent ST was ST10, found in 68 strains (60.7%) out of 112 strains studied, that has also been the main ST reported for *S.* Dublin strains [14–17].

Moreover, other eight STs were detected in the strains studied. Among them, six (ST3734, ST4030, ST4097, ST4100, ST4232, and ST4574) were single-locus variants of ST10, while two (ST4098 and ST4101) were double-locus variants of ST10 (Fig. 1, Table 2). In addition, STs ST4097, ST4098, ST4100, ST4101, ST4232, and ST4574 were detected for the first time in *S.* Dublin strains.

The detection of only single- or double-locus variants of ST10 from clonal complex 10, as well as no prevalence of STs by source, material, or year of isolation among the strains studied, reinforces the proposed by Achtman et al. (2012) that different clonal complexes generally represent specific serovars, mainly due to the highly clonal characteristic of *S. enterica* [15].

Regarding CRISPR and CRISPR-MVLST analysis, the 112 *S.* Dublin strains studied showed 59 CRISPR1 and 12 CRISPR2 and 2 *fimH* and 4 *sseL* different alleles identified, which showed the high differentiation capacity of these methodologies, which were also confirmed by the high values of DI of 0.976 for CRISPR and of 0.980 for CRISPR-MVLST (Figs. 2 and 3, Table 1). However, despite the high discrimination power, the strains showed to be genetically related, with a similarity ≥ 84.4% among the strains for CRISPR and ≥ 84.7 for CRISPR-MVLST (Figs. 2 and 3).

The similar DI found in both methodologies mentioned above showed that the addition of *fimH* and *sseL* in the analysis did not increase the discriminatory power of the methodology on typing *S.* Dublin strains, which differed from previous studies with other *Salmonella* serovars [19, 23–25]. This fact reinforced that this was not due to a technique limitation but to the clonal characteristic of serovar Dublin strains. Similar to MLST, both methodologies grouped the strains independently of geographical, temporal, or isolation source characteristics, which reinforced the idea from previous studies of *Salmonella* Dublin strains from Brazil performed by our research group that suggest that these strains may have descended from a common ancestor that has little differentiated over the years [13].

Table 3 CRISPR-MVLST sequence types (CM-ST) and respective frequencies identified in the 112 *S.* Dublin strains in this study

CM-ST	Number of strains	Alleles			
		CRISPR1	CRISPR2	*fimH*	*sseL*
1	9	53	2	1	1
2	7	5	11	1	1
3	7	43	2	1	1
4	6	5	7	1	1
5	5	5	1	1	1
6	5	29	2	1	1
7	4	35	1	1	1
8	2	5	8	1	1
9	2	5	10	1	1
10	2	10	1	1	1
11	2	36	1	1	1
12	2	49	2	1	1
13	1	1	4	1	1
14	1	2	8	1	1
15	1	3	4	1	1
16	1	4	1	2	1
17	1	4	4	1	1
18	1	4	11	1	2
19	1	5	12	1	1
20	1	5	7	1	3
21	1	6	8	1	1
22	1	6	1	1	1
23	1	7	1	1	1
24	1	8	11	1	1
25	1	9	8	1	1
26	1	11	4	1	1
27	1	12	1	1	1
28	1	13	1	1	1
29	1	14	4	1	1
30	1	15	7	1	1
31	1	16	7	1	1
32	1	17	11	1	1
33	1	18	1	1	1
34	1	19	9	1	1
35	1	20	6	1	1
36	1	21	5	1	1
37	1	22	7	1	1
38	1	23	1	1	1
39	1	24	1	1	1
40	1	25	7	1	1
41	1	26	1	1	1
42	1	27	2	1	1
43	1	28	1	1	1
44	1	30	2	1	1
45	1	31	2	1	4
46	1	32	2	1	1

Table 3 (continued)

CM-ST	Number of strains	Alleles			
		CRISPR1	CRISPR2	*fimH*	*sseL*
47	1	33	1	1	4
48	1	34	1	1	1
49	1	37	1	1	1
50	1	38	1	1	1
51	1	38	1	1	1
52	1	40	1	1	1
53	1	41	1	1	1
54	1	42	1	1	1
55	1	43	2	2	1
56	1	44	2	1	1
57	1	45	2	1	1
58	1	46	2	1	1
59	1	47	2	1	1
60	1	48	2	1	1
61	1	50	2	1	1
62	1	51	2	1	1
63	1	52	2	1	1
64	1	52	3	1	1
65	1	53	1	1	1
66	1	53	2	1	2
67	1	54	2	1	1
68	1	55	2	1	1
69	1	56	2	1	1
70	1	57	2	1	1
71	1	58	2	1	1
72	1	59	1	1	1

In the previous study of our research group [13], the same 112 *S.* Dublin strains of this study were typed by pulsed-field gel electrophoresis (PFGE) and multilocus variable-number tandem repeat analysis (MLVA). Similar to the results found in the present study, PFGE also grouped the strains in a single cluster, with a similarity of ≥ 80.7%. However, that methodology showed a lower discriminatory power, differentiating the strains into 35 PFGE types and with a DI of 0.53. Regarding MLVA, the strains were classified in 89 types with a similarity of ≥23.3% and a DI of 0.95, closer to the results found in the present study. In addition, MLVA was able to group the strains into two different clusters that contained five and 106 strains, respectively, and also two strains showed to be single MLVA types [13].

The DIs observed for the four methodologies mentioned above showed that CRISPR and CRISPR-MVLST are good in silico techniques to type *S.* Dublin strains and alternatives to the non-WGS techniques, such as PFGE and MLVA, considered the gold standard methodologies to type *Salmonella* spp.
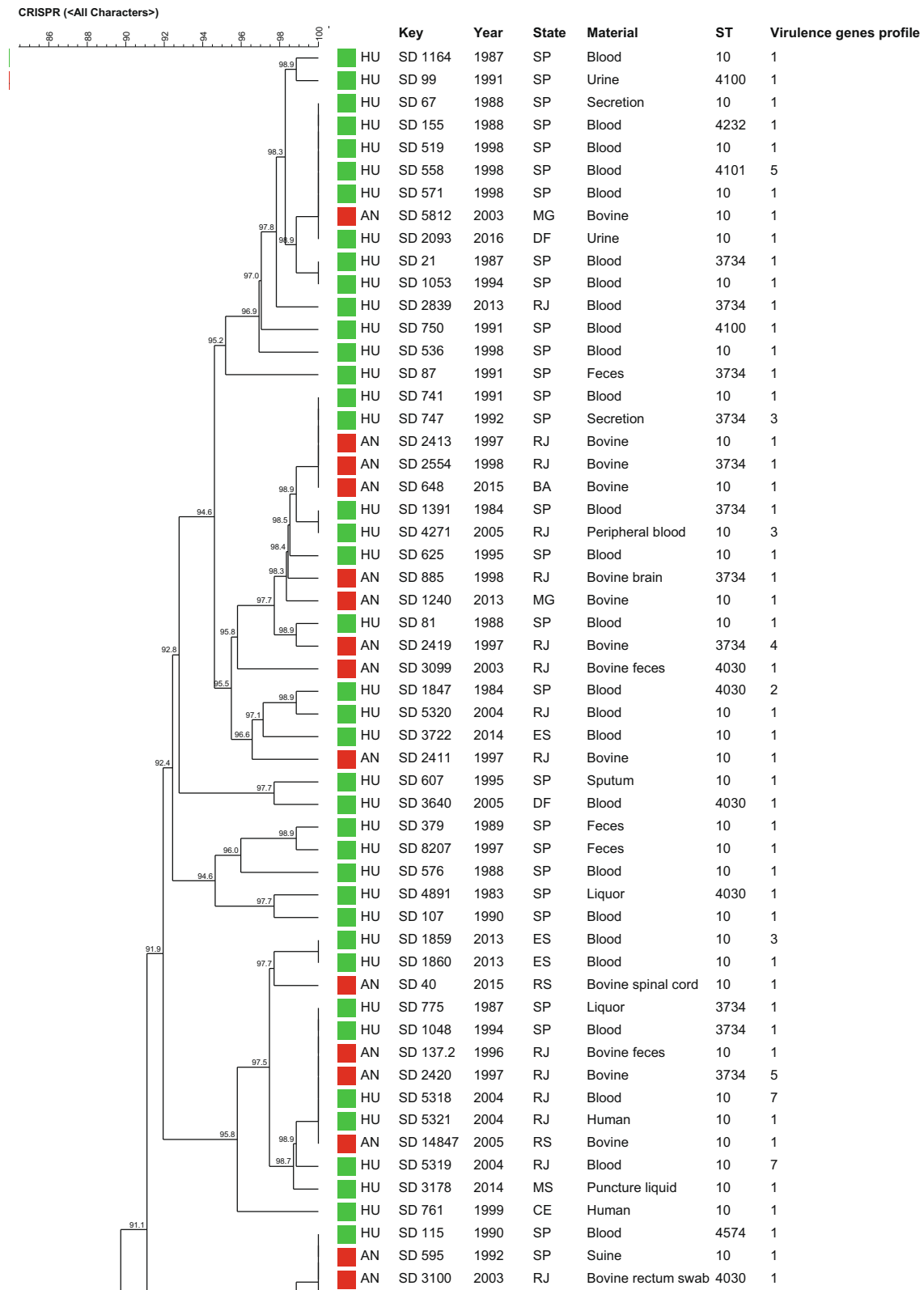
Fig. 2 Similarity dendrogram representing the genetic relationships among *Salmonella* Dublin strains based on the binary matrix of CRISPR1 and CRISPR2 spacers in the 112 strains studied. Green squares represent strains isolated from humans; red squares represent strains isolated from animals. Profile 1 (*fljB* negative), Profile 2 (*fljB* and *spvB* negative), Profile 3 (*fljB*, *spvB*, and *spvC* negative), Profile 4 (*sipA*, *sipD*, *invA*, *hilA*, and *fljB* negative), Profile 5 (*sodCl* and *fljB* negative), Profile 6 (*rpoS* and *fljB* negative), Profile 7 (*sodCl*, *rpoS*, and *fljB* negative)

HU	SD 8924	2003	GO	Blood	10	1
HU	SD 312	1987	SP	Gallbladder	10	1
HU	SD 137	1990	SP	Blood	10	1
HU	SD 749	1991	SP	Peritoneal liquid	4030	1
HU	SD 731	1992	SP	Blood	10	1
AN	SD 2417	1997	RJ	Bovine	3734	1
HU	SD 557	1998	SP	Blood	10	1
AN	SD 2416	1997	RJ	Bovine	3734	1
AN	SD 2418	1997	RJ	Bovine	3734	4
AN	SD 6185	2003	GO	Boar lung	10	1
HU	SD 1030	1994	SP	Blood	10	1
HU	SD 1338	1999	DF	Human	10	1
HU	SD 600	1998	SP	Blood	3734	1
HU	SD 484	1990	SP	Blood	10	1
HU	SD 517	1990	SP	Blood	10	1
AN	SD 2566	1998	MG	Bovine feces	3734	1
AN	SD 3101	2003	RJ	Bovine brain	10	1
HU	SD 8923	2003	GO	Blood	3734	1
HU	SD 1310	2013	MG	Blood	10	1
HU	SD 288	1985	SP	Feces	10	1
HU	SD 3638	2005	DF	Blood	10	1
HU	SD 1032	1993	SP	Blood	3734	1
HU	SD 535	1991	SP	Blood	3734	1
HU	SD 114	1990	SP	Liquor	10	1
HU	SD 530	1990	SP	Blood	10	1
HU	SD 90	1991	SP	Blood	3734	1
HU	SD 534	1991	SP	Liver liquid	3734	1
HU	SD 7362	1993	SP	Liquor	10	1
HU	SD 3639	2005	DF	Hip secretion	4098	1
HU	SD 8731	2010	SP	Blood	10	1
AN	SD 1241	2013	MG	Bovine	10	1
AN	SD 5564	1998	RJ	Bovine liver	4030	3
HU	SD 1883	2013	GO	Blood	10	1
HU	SD 12612	2007	RJ	Urine	4097	1
HU	SD 595.2	1998	SP	Blood	4030	1
HU	SD 903	2005	RJ	Human	10	1
HU	SD 722	1992	SP	Blood	3734	1
HU	SD 8543	2003	SP	Blood	10	3
HU	SD 559	1998	SP	Blood	10	1
HU	SD 290	1985	SP	Blood	3734	1
HU	SD 762	1987	SP	Blood	3734	3
HU	SD 136	1990	SP	Liquor	10	1
AN	SD 2414	1997	RJ	Bovine	10	1
AN	SD 2415	1997	RJ	Bovine feces	10	1
AN	SD 884	1998	RJ	Mouse brain	3734	1
HU	SD 760	1999	CE	Human	10	6
AN	SD 1653	1999	RS	Bovine	10	1
HU	SD 2439	1999	DF	Human	10	1
HU	SD 3179	2014	MS	Puncture liquid	10	1
HU	SD 1019	1993	SP	Urine	4030	1
HU	SD 383	1992	SP	Blood	10	1
HU	SD 1351	1984	SP	Blood	3734	1
AN	SD 883	1998	RJ	Mouse brain	3734	1
AN	SD 1654	1999	RS	Bovine	10	1
HU	SD 1548	2015	RJ	Blood	10	1
AN	SD 647	2015	BA	Bovine	10	1
HU	SD 721	1992	SP	Feces	3734	1

Fig. 2 (continued)

Moreover, CRISPR methodologies are performed in silico, which minimizes reproducibility mistakes among different laboratories.
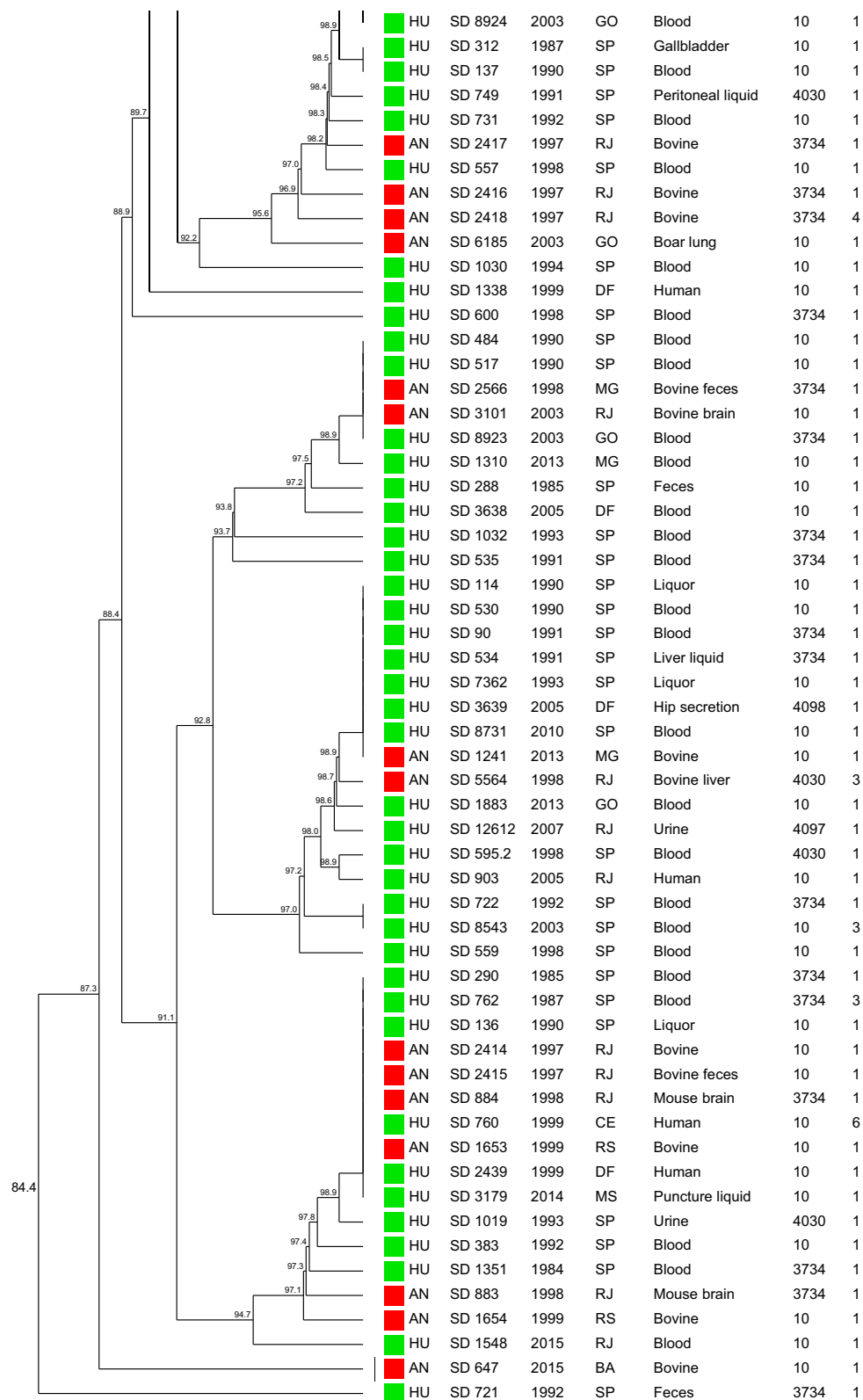
To our knowledge, until the writing of this manuscript, there were no studies performed that used CRISPR and CRISPR-MVLST methodologies to type exclusively *S.*
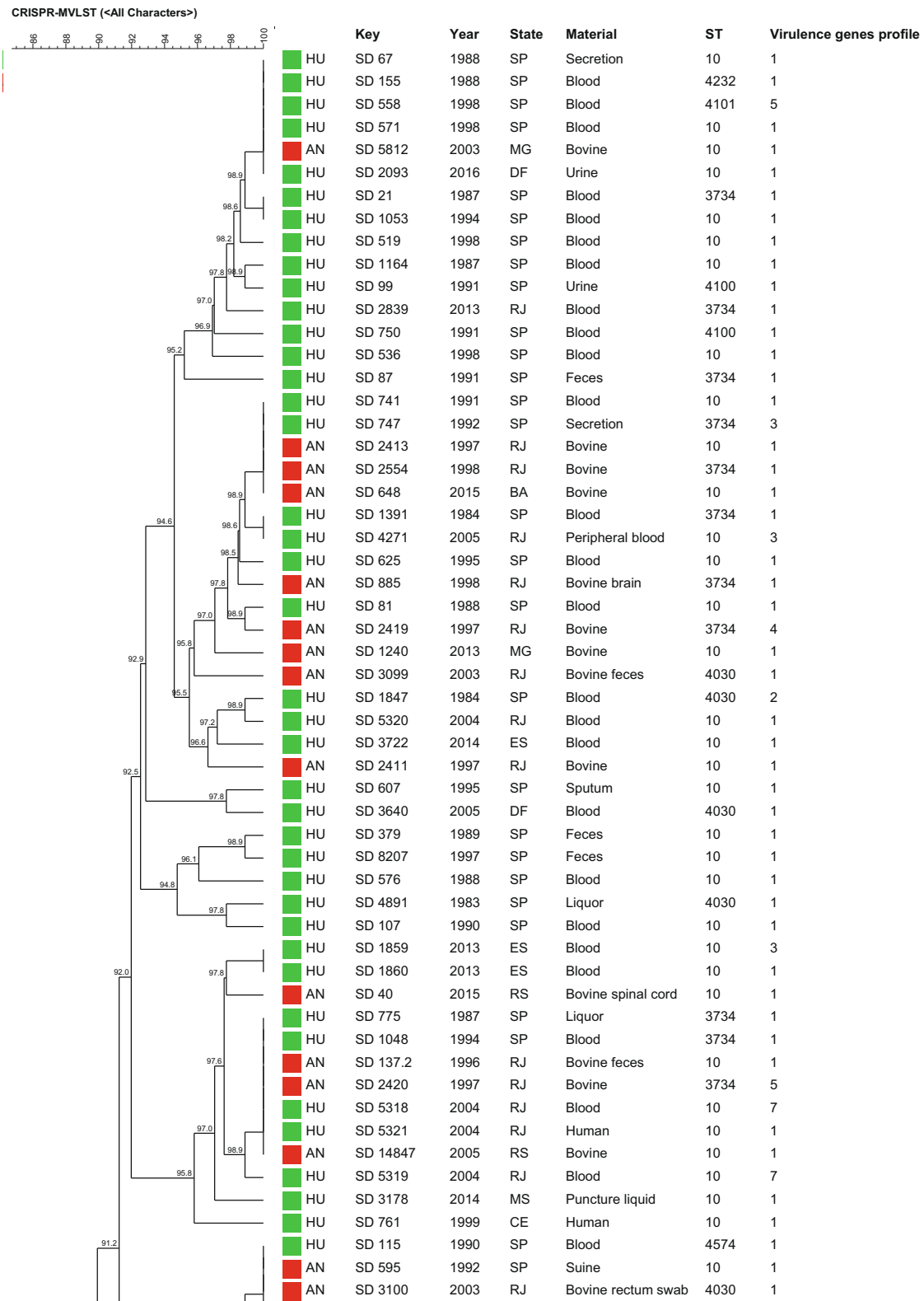
CRISPR-MVLST (<All Characters>)

	Key	Year	State	Material	ST	Virulence genes profile
HU	SD 67	1988	SP	Secretion	10	1
HU	SD 155	1988	SP	Blood	4232	1
HU	SD 558	1998	SP	Blood	4101	5
HU	SD 571	1998	SP	Blood	10	1
AN	SD 5812	2003	MG	Bovine	10	1
HU	SD 2093	2016	DF	Urine	10	1
HU	SD 21	1987	SP	Blood	3734	1
HU	SD 1053	1994	SP	Blood	10	1
HU	SD 519	1998	SP	Blood	10	1
HU	SD 1164	1987	SP	Blood	10	1
HU	SD 99	1991	SP	Urine	4100	1
HU	SD 2839	2013	RJ	Blood	3734	1
HU	SD 750	1991	SP	Blood	4100	1
HU	SD 536	1998	SP	Blood	10	1
HU	SD 87	1991	SP	Feces	3734	1
HU	SD 741	1991	SP	Blood	10	1
HU	SD 747	1992	SP	Secretion	3734	3
AN	SD 2413	1997	RJ	Bovine	10	1
AN	SD 2554	1998	RJ	Bovine	3734	1
AN	SD 648	2015	BA	Bovine	10	1
HU	SD 1391	1984	SP	Blood	3734	1
HU	SD 4271	2005	RJ	Peripheral blood	10	3
HU	SD 625	1995	SP	Blood	10	1
AN	SD 885	1998	RJ	Bovine brain	3734	1
HU	SD 81	1988	SP	Blood	10	1
AN	SD 2419	1997	RJ	Bovine	3734	4
AN	SD 1240	2013	MG	Bovine	10	1
AN	SD 3099	2003	RJ	Bovine feces	4030	1
HU	SD 1847	1984	SP	Blood	4030	2
HU	SD 5320	2004	RJ	Blood	10	1
HU	SD 3722	2014	ES	Blood	10	1
AN	SD 2411	1997	RJ	Bovine	10	1
HU	SD 607	1995	SP	Sputum	10	1
HU	SD 3640	2005	DF	Blood	4030	1
HU	SD 379	1989	SP	Feces	10	1
HU	SD 8207	1997	SP	Feces	10	1
HU	SD 576	1988	SP	Blood	10	1
HU	SD 4891	1983	SP	Liquor	4030	1
HU	SD 107	1990	SP	Blood	10	1
HU	SD 1859	2013	ES	Blood	10	3
HU	SD 1860	2013	ES	Blood	10	1
AN	SD 40	2015	RS	Bovine spinal cord	10	1
HU	SD 775	1987	SP	Liquor	3734	1
HU	SD 1048	1994	SP	Blood	3734	1
AN	SD 137.2	1996	RJ	Bovine feces	10	1
AN	SD 2420	1997	RJ	Bovine	3734	5
HU	SD 5318	2004	RJ	Blood	10	7
HU	SD 5321	2004	RJ	Human	10	1
AN	SD 14847	2005	RS	Bovine	10	1
HU	SD 5319	2004	RJ	Blood	10	7
HU	SD 3178	2014	MS	Puncture liquid	10	1
HU	SD 761	1999	CE	Human	10	1
HU	SD 115	1990	SP	Blood	4574	1
AN	SD 595	1992	SP	Suine	10	1
AN	SD 3100	2003	RJ	Bovine rectum swab	4030	1

Fig. 3 Similarity dendrogram representing the genetic relationships among *Salmonella* Dublin strains based on the combination of the binary matrix of CRISPR1 and CRISPR2 spacers and the loci of *fimH* and *sseL* virulence genes in the 112 strains studied. Green squares represent strains isolated from humans; red squares represent strains isolated from animals. Profile 1 (*fljB* negative), Profile 2 (*fljB* and *spvB* negative), Profile 3 (*fljB*, *spvB*, and *spvC* negative), Profile 4 (*sipA*, *sipD*, *invA*, *hilA*, and *fljB* negative), Profile 5 (*sodCl* and *fljB* negative), Profile 6 (*rpoS* and *fljB* negative), Profile 7 (*sodCl*, *rpoS*, and *fljB* negative)
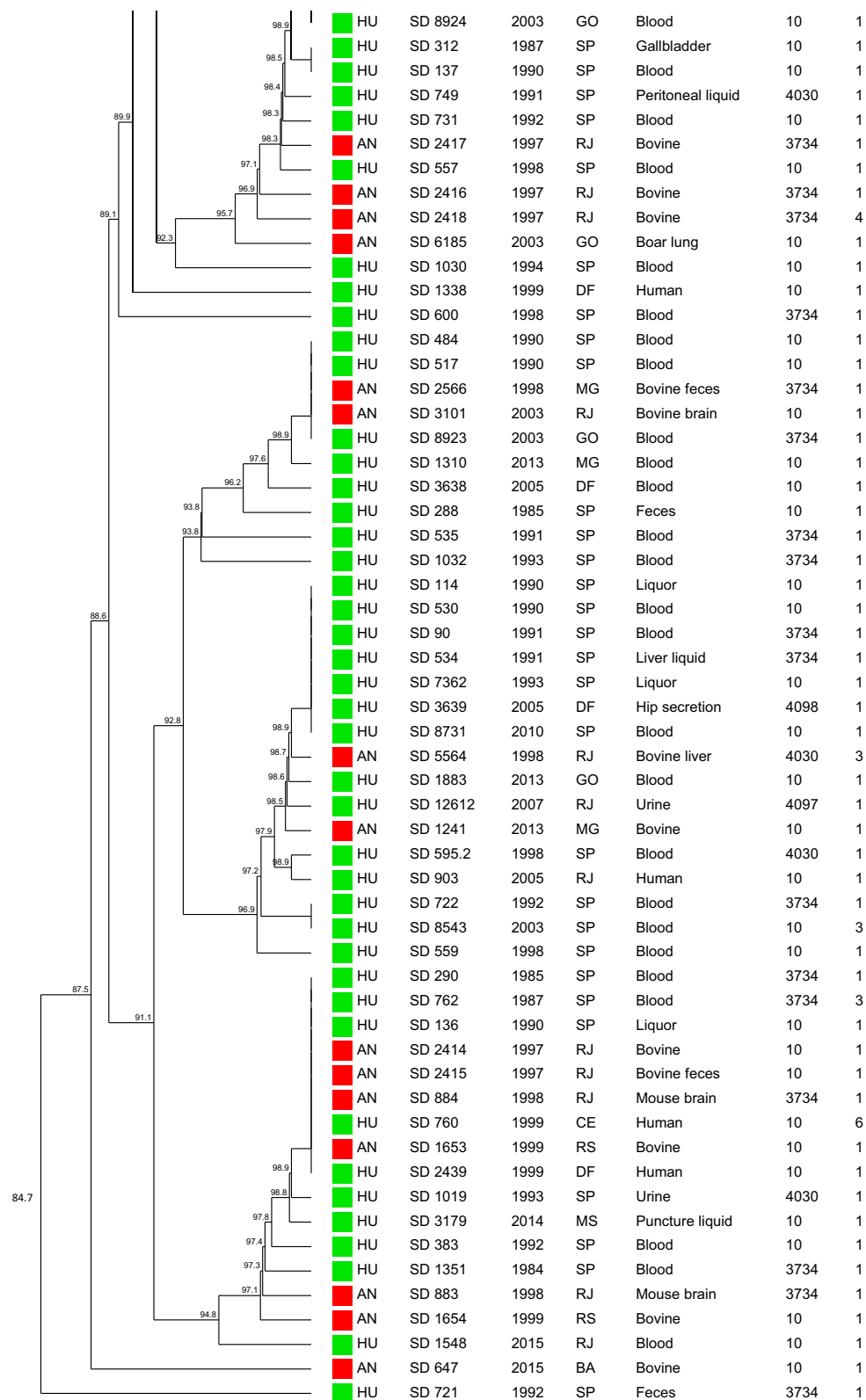
HU	SD 8924	2003	GO	Blood	10	1
HU	SD 312	1987	SP	Gallbladder	10	1
HU	SD 137	1990	SP	Blood	10	1
HU	SD 749	1991	SP	Peritoneal liquid	4030	1
HU	SD 731	1992	SP	Blood	10	1
AN	SD 2417	1997	RJ	Bovine	3734	1
HU	SD 557	1998	SP	Blood	10	1
AN	SD 2416	1997	RJ	Bovine	3734	1
AN	SD 2418	1997	RJ	Bovine	3734	4
AN	SD 6185	2003	GO	Boar lung	10	1
HU	SD 1030	1994	SP	Blood	10	1
HU	SD 1338	1999	DF	Human	10	1
HU	SD 600	1998	SP	Blood	3734	1
HU	SD 484	1990	SP	Blood	10	1
HU	SD 517	1990	SP	Blood	10	1
AN	SD 2566	1998	MG	Bovine feces	3734	1
AN	SD 3101	2003	RJ	Bovine brain	10	1
HU	SD 8923	2003	GO	Blood	3734	1
HU	SD 1310	2013	MG	Blood	10	1
HU	SD 3638	2005	DF	Blood	10	1
HU	SD 288	1985	SP	Feces	10	1
HU	SD 535	1991	SP	Blood	3734	1
HU	SD 1032	1993	SP	Blood	3734	1
HU	SD 114	1990	SP	Liquor	10	1
HU	SD 530	1990	SP	Blood	10	1
HU	SD 90	1991	SP	Blood	3734	1
HU	SD 534	1991	SP	Liver liquid	3734	1
HU	SD 7362	1993	SP	Liquor	10	1
HU	SD 3639	2005	DF	Hip secretion	4098	1
HU	SD 8731	2010	SP	Blood	10	1
AN	SD 5564	1998	RJ	Bovine liver	4030	3
HU	SD 1883	2013	GO	Blood	10	1
HU	SD 12612	2007	RJ	Urine	4097	1
AN	SD 1241	2013	MG	Bovine	10	1
HU	SD 595.2	1998	SP	Blood	4030	1
HU	SD 903	2005	RJ	Human	10	1
HU	SD 722	1992	SP	Blood	3734	1
HU	SD 8543	2003	SP	Blood	10	3
HU	SD 559	1998	SP	Blood	10	1
HU	SD 290	1985	SP	Blood	3734	1
HU	SD 762	1987	SP	Blood	3734	3
HU	SD 136	1990	SP	Liquor	10	1
AN	SD 2414	1997	RJ	Bovine	10	1
AN	SD 2415	1997	RJ	Bovine feces	10	1
AN	SD 884	1998	RJ	Mouse brain	3734	1
HU	SD 760	1999	CE	Human	10	6
AN	SD 1653	1999	RS	Bovine	10	1
HU	SD 2439	1999	DF	Human	10	1
HU	SD 1019	1993	SP	Urine	4030	1
HU	SD 3179	2014	MS	Puncture liquid	10	1
HU	SD 383	1992	SP	Blood	10	1
HU	SD 1351	1984	SP	Blood	3734	1
AN	SD 883	1998	RJ	Mouse brain	3734	1
AN	SD 1654	1999	RS	Bovine	10	1
HU	SD 1548	2015	RJ	Blood	10	1
AN	SD 647	2015	BA	Bovine	10	1
HU	SD 721	1992	SP	Feces	3734	1

Fig. 3 (continued)

Dublin strains, which makes the comparison of the results found in the present study difficult. However, other studies using these methodologies had been successfully conducted to subtype other *Salmonella* serovars, such as Typhimurium, Newport, Enteritidis, and Heidelberg [19, 23–25].

The presence of multiple virulence genes was verified in the strains studied (Table 1). The high prevalence of SPI-1 and SPI-2 genes detected in the *S.* Dublin strains studied reinforced the invasive potential and the capacity to cause serious disease of strains of this serovar [8, 36–38]. The absence of the genes *spvB* and *spvC* in seven and six strains studied, respectively, may be explained to a possible absence of *pSDL2*, a well-characterized *S. enterica* virulence plasmid encoded by genes of *spv* locus [39, 40].

Regarding the flagella-related genes, the *fljB*, responsible for phase-2 flagellin, was absent in all the strains studied, while *fliC* gene, responsible for phase-1 flagellin, was found in all the strains studied, which showed a prevalence of this type of flagella among the strains studies. It is interesting to mention that Yim et al. (2014) [16] showed differences in the expression of *fliC* gene in *S.* Dublin strains from Uruguay that may alter the flagella expression [16].

Although some genes such as *hilA* and *rpoS* are important transcriptional regulators and mainly detected in *Salmonella*, in this study, these genes were not detected in two and three of the strains studied, respectively. Previous studies have already reported the absence of *hilA* in *Salmonella* serovars [41, 42]. The absence of *rpoS* might have been due to a genome assembly drawback, which may have led to a non-detection of any gene fragment below the parameters established for the detection of this gene.

In conclusion, the high similarity among the strains reinforces the clonal nature of the strains of this serovar that may have descended from a common ancestor that little differed over 33 years in Brazil. CRISPR and CRISPR-MVLST showed to be good alternatives to type *S.* Dublin strains. MLST suggested that *S.* Dublin strains from Brazil were phylogenetically related to strains from other parts of the globe. Moreover, the high frequency of virulence genes among the strains studied reinforces the capacity of *S.* Dublin to cause invasive diseases.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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