

# New Sequence Types and Antimicrobial Drug–Resistant Strains of *Streptococcus suis* in Diseased Pigs, Italy, 2017–2019

## Appendix 2

### Read quality, number of contigs and coverage of Whole-Genome Sequencing data

The final assembly of the 78 sequences resulted in an average read quality after trimming of 34.60% (min 34.04, max 34.89) and 3,165,852 read pairs (min 1,043,985; max 5,077,416). The average number of contigs was 68 (min 25, max 177), with a mean length of 2,114,359 nucleotides (min 2,026,262; max 2,264,709). The average vertical coverage was 211 (min, 69; max, 339). The mean N50 and L50 values were 149,502 nucleotides (min 371, 66; max 293,044) and 6.95 (min 3, max 21), respectively.

### References

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**Appendix 2 Table 1.** Primers used to identify the *S. suis* serotypes and virulence factors by PCR\*

Target	Primer sequences (5' to 3')		Reference
	Forward	Reverse	
<b>For grouping PCR</b>			
I	TGGTTCAAATATCAATGCTC	ATTGGTTGTGAGTGCATTG	(1)
II	TCAAAATACGCACCTAAGGC	CACTCACCTGCCCAAGAC	
III	TGATTTGGGTGAGACCATG	CTCATGCTGGATAACACGT	
IV	ACAGTCGGTCAAGATAATCG	TCAGCTTGGGTAATATCTGG	
V	GGAAAGATGGAGGACCAGC	CCAACCAGACTCATATCCCC	
III and VI	GATGCCCCAAGCGATATGCC (F1) GACGCACCAAGTGATATGCC (F2)	GGACCAACAATGGCCATCTC (R1) GGTCCGACAATAGCCATTTT (R2)	
<b>For typing PCR</b>			
SS group I			(1)
3	GGTTTTGATTGGTCTAGTTG	CTCTAAAGCTCGATATCTAC	
13	TATGGTTAAAGGTGGAAGCTG	CCTTGATATATTTCCCTCCA	
18	TAATGGGATAGTTGCCATTAC	ATACATAAAGTTGTCCTGCG	
SS group II			(1)
2	TTAGCAACGTTGCCAATAAG	AATCCTCCATTAACAAACCCTG	
6	GCTCACTATTTTTACATTACAC	TATTAATCCGCCAAATACAG	
1 and 14	TTAGACAGACACCTTATAGG	CTAGCTTCGTTACTTGTATTC	
16	AAGGTTATCCACGAAAGATG	TCCGGCAATATTTCTTTCAAG	
27	AGACACTGCTTGCATTATTG	TCAGAATTACTTCTGTGTTGC	
1 and 1/2	ATCGCTTTGTGGTGGCCCTT	AGAAGCTTCTTTTGTGTTTGC	(2)
1 and 14	ATCGCTTTGTGGTGGCCCTT		
SS group III			(1)
21	TATCATATTGAGAATCTTCCC	TTGCGTAGCATACAAAGTTC	
28	ATTATGTTGGTTGCAGAAGG	CGACTCAATTGTTGTAGTAG	
29	TTCTGGGATTTTAGGAATGC	CATGAAATACGCACCTTGTAC	
30	TATTGCACTAGCTTCAGAAC	TGCATCCATAGTTGTATTCC	
SS group IV			(1)
4	GACTATCTGTATACCCAAAC	TCCTTCCAAGTATTCTCTAG	
5	ATCTTAGGAATGATTCGGAC	ACCAGATATCTGAGCAAATG	
7	AACTACCTACCTGAACCTTG	AGTCTAAAAGTGATCGAGTC	
17	TAGCATCAGTTTATACGAGG	TAGTTTATCTGTGACACACC	
19	GTGTCGCAAATCAAGTATTG	AAGCTAGTACAACAAGCATG	
23	TAATGTATGCTCTGTCACTG	AACGAAACGGAATAGTTTGC	
SS group V			(1)
8	AAATAAGGTAGGAGCTACTC	ATCCAACCTTAGCTTTCTGT	
15	ATCGTTTTGAGATTGAGTGG	TAAACGGATTCCGGTACTCA	
20	TGTGGATTTCTGGGATAATC	TGTGGACGAATTACTACTTG	
22	GCATTATCAGGATTCTTTCC	CCAATTGGGTGTTCAAAAAG	
25	GTTTGCTCCGATCATAATAG	CCAGTAAAAGGACTCAATAC	
SS group VI			(1)
9	GAAAGTAGGTATATCTCAGC	GGGCTATTAACCTCCTATC	
10	TTTCCCATTTGCTTATGGAC	GGAATAAAAACGATTGGGAG	
11	ATGCGATTGCAACAATTGAC	AGGCATGAGTAATACATAGG	
12	AACAGGTATTTCAAGATTGC	CTCGGATAAAGATAATCAGC	
24	TACTGAGATTTATTGGGACG	AAGCGATTGGATTACATTGC	
26	TTATACCGAAATTTGTTGCC	CGTCAATCATATAAAGTGGG	
33	GATGTTTTCAACAGGTGTAC	CAAAGTACCTATTTTCAGCG	
SS group VII			(1)
31	ACAATCGTTTCTGCAATACG	GATGAAAACATCGTTGGTAG	
	ATCAGTAGTGGGAATAGTTG	TTTACTGTTTTTCGACCGTG	
32	AACCGCTGTTGAATTAAGAG	TTCGTTAGTTGAAGTGTTC	
	TAGGACTATGGTTCCTAATG	TATTCTAGTTCAAGTCGCTC	
34	AAGTTTCATTCGAGGACTTC	GTATATAACACCGCAAGAAG	
	ATACAGTGATGTCTTGCAAC	ATTGCTTTTTGACAATCGGC	
<b>For virulence factors</b>			
mrp	GACAGATGGTGAGGAAAATGG	TGAGCTTTACCTGAAGCGGT	(3)
epf	GCTACGACGGCCTCAGAAATC	TGGATCAACCACTGGTGTAC	(4)
Sly	CAGCTCGTTGCCCTGTACTA	ACTCTATCACCTCATCCGC	(5)

\*The DNA for SS3 and SS14 were kindly provided by Marcelo Gottschalk (University of Montreal, Canada) and used as positive control. *S. suis* strain V20 from a previous study conducted in Italy (6) was used as control.

**Appendix 2 Table 2.** Genes lacking in Lineage III and Lineage I ST7 reference strains, but present in the ST7 isolates of this study

Gene	Annotation
group_16	putative autolysin SsaALP
group_2145	IS630 family transposase ISSsu3
elaA	Protein ElaA
group_324	hypothetical protein
group_325	hypothetical protein
group_326	hypothetical protein
group_327	hypothetical protein
pdxK	Pyridoxine kinase
hmpT	Thiamine precursor transporter HmpT
group_330	hypothetical protein
group_331	hypothetical protein
baeS	Signal transduction histidine-protein kinase BaeS
walR_2	Transcriptional regulatory protein WalR
group_334	hypothetical protein
mco	Multicopper oxidase mco
group_336	IS3 family transposase ISStin7
group_337	hypothetical protein
group_338	hypothetical protein
group_339	hypothetical protein
group_340	hypothetical protein
immR	HTH-type transcriptional regulator ImmR
mhqD	Putative hydrolase MhqD
mhqA	Putative ring-cleaving dioxygenase MhqA
pgl_1	6-phosphogluconolactonase
group_347	hypothetical protein
lgt_1	Phosphatidylglycerol--prolipoprotein diacylglyceryl transferase
group_349	hypothetical protein
group_350	hypothetical protein
copB	Copper-exporting P-type ATPase B
group_352	hypothetical protein
actP	Copper-transporting P-type ATPase
copY_2	Transcriptional repressor CopY
cadC_1	Cadmium resistance transcriptional regulatory protein CadC
group_356	hypothetical protein
group_357	hypothetical protein
group_358	hypothetical protein
group_359	hypothetical protein
group_360	hypothetical protein
cadC_2	Cadmium resistance transcriptional regulatory protein CadC
cadA	putative cadmium-transporting ATPase
yadH	Inner membrane transport permease YadH
yadG	putative ABC transporter ATP-binding protein YadG
cdr_1	Coenzyme A disulfide reductase
acr3	Arsenical-resistance protein Acr3
arsA	Arsenical pump-driving ATPase
group_368	hypothetical protein
arsD	Arsenical resistance operon trans-acting repressor ArsD
group_37	Deoxyguanosinetriphosphate triphosphohydrolase-like protein
group_370	IS6 family transposase IS1216V
group_371	hypothetical protein
group_372	hypothetical protein
dnaG_1	DNA primase
group_374	hypothetical protein
group_375	hypothetical protein
group_376	hypothetical protein
group_377	hypothetical protein
ltrA	Group II intron-encoded protein LtrA
group_379	hypothetical protein
group_380	hypothetical protein
group_381	hypothetical protein
group_382	hypothetical protein
group_383	hypothetical protein
group_45	hypothetical protein
yeeO_3	putative FMN/FAD exporter YeeO
group_82	hypothetical protein
sdpR_1	Transcriptional repressor SdpR

**Appendix 2 Table 3.** Genes lacking in the ST7 isolates of this study, but present in Lineage I ST7 reference strains

Gene	Annotation
group_310	Tyrosine recombinase XerC
group_311	hypothetical protein
group_312	hypothetical protein
group_313	hypothetical protein
group_314	hypothetical protein
group_315	hypothetical protein
group_316	hypothetical protein
group_317	hypothetical protein
group_318	hypothetical protein
group_319	hypothetical protein
group_320	hypothetical protein
group_321	hypothetical protein
group_22	hypothetical protein
group_395	hypothetical protein
group_396	Adaptive-response sensory-kinase SasA
group_397	Response regulator ArlR
group_398	hypothetical protein
group_399	hypothetical protein
yxIF_2	putative ABC transporter ATP-binding protein YxIF
lmrA	Multidrug resistance ABC transporter ATP-binding and permease protein
group_402	hypothetical protein
group_403	hypothetical protein
group_404	hypothetical protein
group_405	hypothetical protein
group_406	hypothetical protein
group_407	hypothetical protein
group_408	hypothetical protein
group_409	hypothetical protein
Int-Tn	Transposase from transposon Tn916
group_411	hypothetical protein
group_412	hypothetical protein
group_413	hypothetical protein
group_414	hypothetical protein
tet(M)	tetracycline resistance ribosomal protection protein Tet(M)
group_416	hypothetical protein
group_417	hypothetical protein
group_418	hypothetical protein
group_419	hypothetical protein
group_420	hypothetical protein
group_421	hypothetical protein
group_422	hypothetical protein
group_423	hypothetical protein
group_424	hypothetical protein
group_425	hypothetical protein
group_426	hypothetical protein
pcrA_2	ATP-dependent DNA helicase PcrA
group_428	hypothetical protein
pezT	Toxin PezT
group_430	hypothetical protein
group_431	DNA primase
degU	Transcriptional regulatory protein DegU
group_433	hypothetical protein
group_434	hypothetical protein
group_435	hypothetical protein
bcrA_2	Bacitracin transport ATP-binding protein BcrA
lagD	Lactococcin-G-processing and transport ATP-binding protein LagD
group_438	hypothetical protein
group_439	hypothetical protein
group_440	hypothetical protein
group_441	hypothetical protein
group_442	hypothetical protein
group_443	hypothetical protein
group_444	2-methoxy-6-polyprenyl-1,4-benzoquinol methylase, mitochondrial
aadK	Aminoglycoside 6-adenylyltransferase
apt_1	Adenine phosphoribosyltransferase
group_447	IS1380 family transposase ISSu5

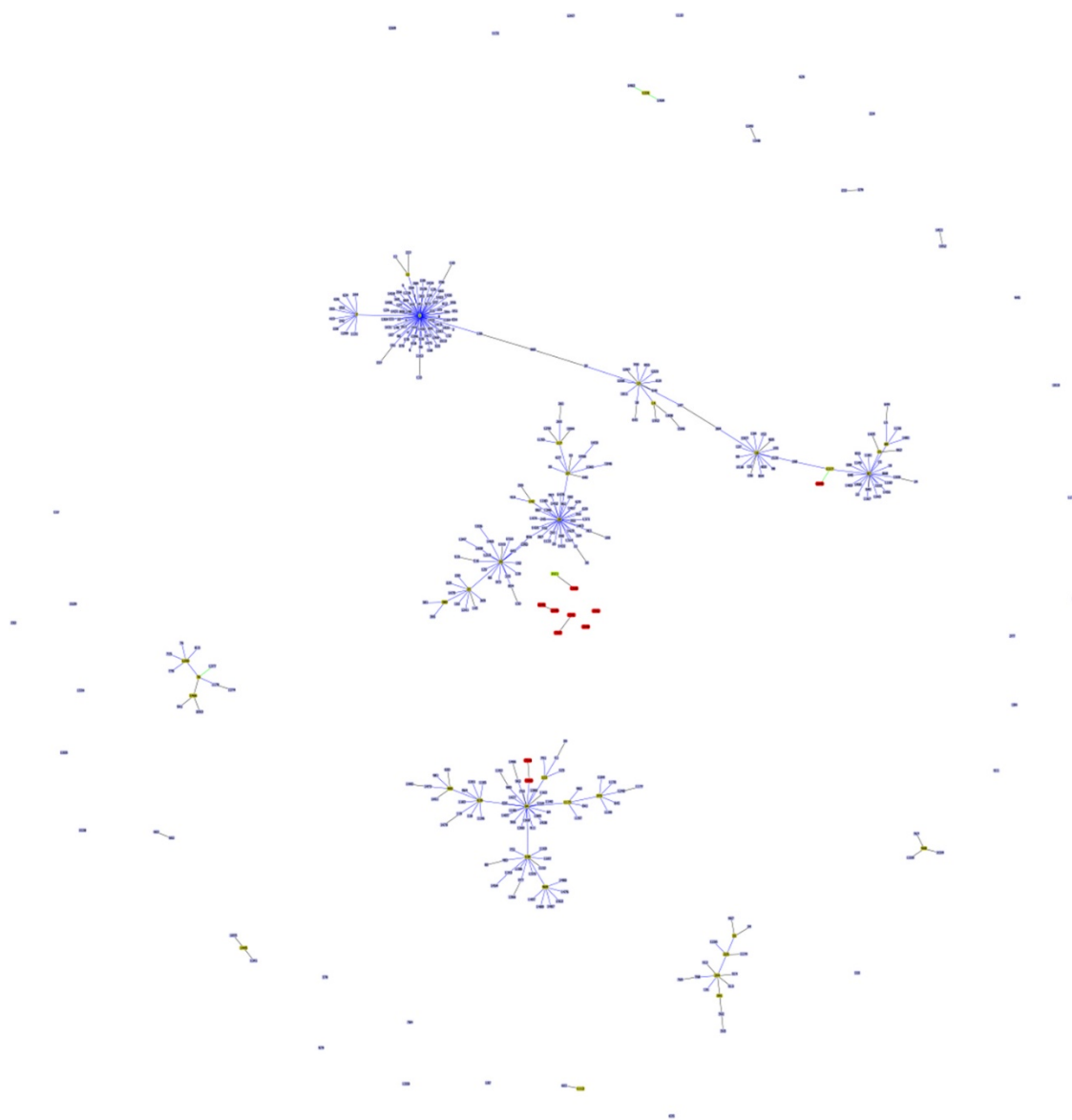
Gene	Annotation
group_448	hypothetical protein
group_449	Lantibiotic macedovicin
group_450	hypothetical protein
group_451	hypothetical protein
group_452	hypothetical protein
group_453	hypothetical protein
glgP	Glycogen phosphorylase

**Appendix 2 Table 4.** Genes lacking in the ST7 isolates of this study, but present in Lineage III ST7 reference strains

Gene	Annotation
group_173	hypothetical protein
group_127	hypothetical protein
group_179	hypothetical protein
group_18	hypothetical protein
entS	Enterobactin exporter EntS
msr(D)	ABC-F type ribosomal protection protein Msr(D)
group_182	hypothetical protein
group_183	hypothetical protein
dinB_2	DNA polymerase IV
group_185	hypothetical protein
group_186	hypothetical protein
group_187	hypothetical protein
group_188	hypothetical protein
group_189	hypothetical protein
group_190	hypothetical protein
group_191	hypothetical protein
group_192	hypothetical protein
group_193	hypothetical protein
group_194	hypothetical protein
group_195	hypothetical protein
group_196	hypothetical protein
group_197	hypothetical protein
group_198	hypothetical protein
group_199	hypothetical protein
group_200	hypothetical protein
metK_1	S-adenosylmethionine synthase
group_202	hypothetical protein
group_203	hypothetical protein
group_204	hypothetical protein
group_205	hypothetical protein
group_206	hypothetical protein
group_207	hypothetical protein
group_208	hypothetical protein
group_209	hypothetical protein
group_210	hypothetical protein
group_211	hypothetical protein
group_212	hypothetical protein
group_213	hypothetical protein
group_214	hypothetical protein
group_215	hypothetical protein
clpP_1	ATP-dependent Clp protease proteolytic subunit
group_217	hypothetical protein
group_218	hypothetical protein
group_219	hypothetical protein
group_220	hypothetical protein
group_221	hypothetical protein
group_222	hypothetical protein
group_223	hypothetical protein
smc_1	Chromosome partition protein Smc
group_225	hypothetical protein
group_226	hypothetical protein
group_227	hypothetical protein
group_228	hypothetical protein
group_230	hypothetical protein
group_231	hypothetical protein
group_232	hypothetical protein
group_233	hypothetical protein
group_234	hypothetical protein

Gene	Annotation
group_235	hypothetical protein
aphA	Aminoglycoside 3'-phosphotransferase
satA	Streptothricin acetyltransferase A
group_238	Aminoglycoside 6-adenylyltransferase
group_239	hypothetical protein
group_241	hypothetical protein
group_242	hypothetical protein
group_243	hypothetical protein
group_244	hypothetical protein
group_245	hypothetical protein
group_250	hypothetical protein
group_251	hypothetical protein
group_253	hypothetical protein
group_254	hypothetical protein
group_255	hypothetical protein
group_256	hypothetical protein
group_257	hypothetical protein
group_258	hypothetical protein
noc	Nucleoid occlusion protein
group_260	hypothetical protein
group_261	hypothetical protein
group_262	hypothetical protein
group_263	hypothetical protein
group_264	hypothetical protein
group_265	DNA primase
group_267	Toxin PezT
group_268	hypothetical protein
group_269	hypothetical protein
group_270	hypothetical protein
group_271	hypothetical protein
group_272	hypothetical protein
nisP	Nisin leader peptide-processing serine protease NisP
regX3	Sensory transduction protein regX3
creC	Sensor protein CreC
bcrA_1	Bacitracin transport ATP-binding protein BcrA
group_277	hypothetical protein
group_278	hypothetical protein
nsuA	Lantibiotic nisin-U
nisB	Nisin biosynthesis protein NisB
group_281	putative ABC transporter ATP-binding protein
nisC	Nisin biosynthesis protein NisC
nisI_1	Nisin immunity protein
group_284	hypothetical protein
group_289	hypothetical protein
group_292	hypothetical protein
group_293	hypothetical protein
group_294	hypothetical protein
group_295	PTS system mannose-specific EIID component
sorC_2	PTS system sorbose-specific EIIC component
sorB_2	PTS system sorbose-specific EIIB component
group_298	hypothetical protein
group_299	hypothetical protein
xylB	Xylulose kinase
xylA	Xylose isomerase
nagC	N-acetylglucosamine repressor
group_303	ISL3 family transposase ISSsu13
group_304	hypothetical protein
group_32	IS110 family transposase ISCARN28
group_71	hypothetical protein
group_78	hypothetical protein
gmuD_3	6-phospho-beta-glucosidase GmuD
group_15	hypothetical protein
purD_2	Phosphoribosylamine--glycine ligase
group_290	hypothetical protein
group_3	IS110 family transposase ISSsu7
group_31	IS110 family transposase ISCARN28
group_34	IS110 family transposase ISCARN28
group_35	IS110 family transposase ISCARN28
group_39	hypothetical protein

Gene	Annotation
mraY_1	Phospho-N-acetylmuramoyl-pentapeptide-transferase
group_4	IS110 family transposase ISSsu7
group_43	hypothetical protein
glf_1	UDP-galactopyranose mutase
group_61	hypothetical protein
group_65	hypothetical protein
group_67	hypothetical protein
group_7	IS110 family transposase ISSsu7
group_96	IS4 family transposase ISSsu2



**Appendix 2 Figure 1.** e-BURST illustration of the *Streptococcus suis* population was used together with the complete MLST database to cluster the sequence types (STs) into major clonal complexes (CCs). The representation of groups was performed with the double-locus variants (DLVs) parameters. Primary founders (green) are positioned at the center of the cluster and subgroup founders are showed in yellow. The new STs described in our study are highlighted in red.





**Appendix 2 Figure 2.** Results of comparison between the genomes of our ST7 with the genomes of CS100322 and SC070731, two novel ST7 types described by Dong et al. (7) as belonging to lineage III, with SC84 and SC19, two ST7 lineage I strains, and with P1-7 as reference strain. The figure was generated using Phandango, an interactive viewer for bacterial population genomics (<https://jameshadfield.github.io/phandango/#/>) using as input the file generated by Roary (gene\_presence\_absence.csv and accessory\_binary\_genes.fa.newick). Genes are shown as light blue bricks along the top and are sorted left to right by the proportion of isolates they are observed in. Presence (blue) and absence (white) of genes are plotted considering the phylogenetic placement of each isolates.

