



**HAL**  
open science

## Genomic Insights into Methicillin-Resistant Staphylococcus aureus spa Type t899 Isolates Belonging to Different Sequence Types

Henok Ayalew Tegegne, Ivana Koláčková, Martina Florianová, Pierre Wattiau,  
Tereza Gelbíčová, Cécile Boland, Jean-Yves Madec, Marisa Haenni, Renáta  
Karpíšková

### ► To cite this version:

Henok Ayalew Tegegne, Ivana Koláčková, Martina Florianová, Pierre Wattiau, Tereza Gelbíčová, et al..  
Genomic Insights into Methicillin-Resistant Staphylococcus aureus spa Type t899 Isolates Belonging  
to Different Sequence Types. Applied and Environmental Microbiology, 2021, 87 (6), pp.e01994-20.  
10.1128/aem.01994-20 . anses-03987672

**HAL Id: anses-03987672**

**<https://anses.hal.science/anses-03987672v1>**

Submitted on 14 Feb 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License



# Genomic Insights into Methicillin-Resistant *Staphylococcus aureus spa* Type t899 Isolates Belonging to Different Sequence Types

Henok Ayalew Tegegne,<sup>a,b,c,d</sup> Ivana Koláčková,<sup>a</sup> Martina Florianová,<sup>a</sup> Pierre Wattiau,<sup>c</sup> Tereza Gelbíčová,<sup>a</sup> Cécile Boland,<sup>c</sup> Jean-Yves Madec,<sup>d</sup> Marisa Haenni,<sup>d</sup> Renáta Karpíšková<sup>a,b</sup>

<sup>a</sup>Department of Bacteriology, Veterinary Research Institute, Brno, Czech Republic

<sup>b</sup>Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic

<sup>c</sup>Veterinary Bacteriology, Sciensano, Brussels, Belgium

<sup>d</sup>Unité Antibiorésistance et Virulence Bactériennes, University of Lyon-ANSES, Lyon, France

Marisa Haenni and Renáta Karpíšková contributed equally to this work. Author order was determined alphabetically.

**ABSTRACT** Methicillin-resistant *Staphylococcus aureus* (MRSA) presenting *spa* type t899 is commonly associated with sequence type 9 (ST9) but is also increasingly linked to ST398. This study provides genomic insight into the diversity of t899 isolates using core genome multilocus sequence typing (cgMLST), single nucleotide polymorphism (SNP)-based phylogeny, and the description of selected antimicrobial resistance and virulence markers. The SNP-based phylogenetic tree showed that isolates sharing the same *spa* type (t899) but different STs highly diverged in their core and accessory genomes, revealing discriminant antimicrobial resistance (AMR) and virulence markers. Our results highlighted the idea that in a surveillance context where only *spa* typing is used, an additional multiplex PCR for the detection of the *tet(M)*, *sak*, and *seg* genes would be valuable in helping distinguish ST9 from ST398 isolates on a routine basis.

**IMPORTANCE** This study showed the genetic diversity and population structure of *S. aureus* presenting the same *spa* type, t899, but belonging to different STs. Our findings revealed that these isolates vary deeply in their core and accessory genomes, contrary to what is regularly inferred from studies using *spa* typing only. Given that identical *spa* types can be associated with different STs and that *spa* typing only is not appropriate for *S. aureus* isolates that have undergone major recombination events which include the passage of the *spa* gene (such as in t899-positive MRSA), the combination of both MLST and *spa* typing methods is recommended. However, *spa* typing alone is still largely used in surveillance studies and basic characterization. Our data suggest that additional markers, such as *tet(M)*, *sak*, and *seg* genes, could be implemented in an easy and inexpensive manner in order to identify *S. aureus* lineages with a higher accuracy.

**KEYWORDS** MRSA, t899, *spa* type, MLST, cgMLST, whole-genome sequencing

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) was first described in 2005 (1, 2) and rapidly gained importance because of its capacity to colonize and infect humans, particularly pig farmers. Clonal complex 398 (CC398) has been referred to as the predominant LA-MRSA lineage in Europe (3, 4), but other lineages, including CC9 and CC5, have also been detected (3–7).

*spa* type t899 originally belongs to CC9 (sequence type 9 [ST9]), the predominant LA-MRSA genotype reported in Asia, and was identified in pigs and their associated human workers (8). More recently, *spa* type t899 was also reported in a CC9/CC398 hybrid strain in Europe (9, 10). This hybrid is a unique genotype with a CC398 chromosomal backbone

**Citation** Tegegne HA, Koláčková I, Florianová M, Wattiau P, Gelbíčová T, Boland C, Madec J-Y, Haenni M, Karpíšková R. 2021. Genomic insights into methicillin-resistant *Staphylococcus aureus spa* type t899 isolates belonging to different sequence types. Appl Environ Microbiol 87:e01994-20. <https://doi.org/10.1128/AEM.01994-20>.

**Editor** Johanna Björkroth, University of Helsinki

**Copyright** © 2021 Tegegne et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Henok Ayalew Tegegne, [henokayalew79@gmail.com](mailto:henokayalew79@gmail.com).

**Received** 15 August 2020

**Accepted** 14 December 2020

**Accepted manuscript posted online** 4 January 2021

**Published** 26 February 2021

and smaller CC9 region with a *spa* gene (9). CC9 differs from the European pig-associated CC398 with regard to clonal type, staphylococcal cassette chromosome *mec* element (SCC*mec*) content, and resistance profile (4).

CC398 (ST398) isolates belonging to *spa* type t899 have received specific attention because they cause sporadic illness in humans (9, 11–13). This lineage notably harbors the staphylococcal complement inhibitor (*scn*), staphylokinase (*sak*), and chemotaxis inhibitory protein (*chp*) genes associated with the  $\phi$ Sa3 immune evasion cluster (IEC), allowing adaptation to the human host (10). *spa* types t011 and t034 (which display close tandem repeat successions) are still the main types associated with CC398, but t899 is increasingly described in the literature both in human and veterinary medicine (14–19).

The CC9/CC398 hybrid strain has been identified from livestock (9, 10) and related personnel (11, 20) in several European countries. Furthermore, two CC398 LA-MRSA *spa* type t899 isolates were recently reported as incidental findings during a clinical investigation from turkey and pheasant in the United Kingdom (10, 21). These two isolates were shown to belong to the CC9/C398 hybrid genotype and were quite similar to the clone that was reported from continental Europe (10, 13, 22). Interestingly, t899 has been increasingly associated with several single-locus variants (SLVs) of CC398 and CC9 (16–18).

Overall, the occurrence of the same *spa* types in distant lineages has been reported, resulting from either convergent evolution or genetic recombination (23, 24). As an example, CC239 MRSA is a hybrid strain of CC30 (founder, ST30) and CC8 (founder, ST8) (23). ST34 and ST42 backgrounds have also been suggested to be of hybrid origin (24). Recently, t304 isolates belonging to ST6, ST1649, ST8, and ST4290 were reported by Bartels et al. (25).

Since *spa* typing is still largely used as the unique typing method, for example in large surveillance studies and in low-income countries, our aim was to characterize t899 isolates using single nucleotide polymorphism (SNP)-based phylogeny on publicly available genomic data and associated metadata in order to identify markers that could be implemented in an easy and inexpensive manner in order to identify LA-MRSA lineages with a higher accuracy.

## RESULTS

Thirty-four LA-MRSA genomes of t899 isolates were analyzed, of which 20 belonged to ST398, 13 to ST9, and 1 to ST4034 (a single-locus variant [SLV] of ST398, differing by one substitution [A<sub>294</sub>T] in the *arcC* gene). Metadata associated with the selected isolates were recorded (Table 1). All t899 isolates harbored the *mecA* gene on a SCC*mec* IVa(2B) element, except for two which presented either the SCC*mec* V element or an undefined cassette. Both the spanning tree and the SNP-based phylogenetic tree (Fig. 1B and 2) confirmed a strong clustering according to the ST, with the ST4034/t899 isolate differing from ST398/t899 by only 41 core alleles. Other characteristics, including matrix/sample origin, did not appear to cluster in this SNP analysis.

The phylogenetic tree (Fig. 2) also showed major divergences in the antimicrobial resistance and virulence patterns depending on the ST. All ST398 isolates carried the *tet(M)* gene, while none of the ST9 isolates carried this tetracycline resistance gene (Fig. 2). Concerning virulence markers, most ST398 *spa* type t899 isolates harbored the *scn* and *sak* genes, indicating the presence of the IEC cluster, while ST9 isolates were devoid of the IEC cluster but systematically harbored the *seg*, *sei*, *sem*, *sen*, and *seu* genes, encoding enterotoxin-like proteins (Fig. 2).

## DISCUSSION

In this study, the SNP-based phylogeny analysis was consistent with the core genome multilocus sequence typing (cgMLST) analysis, with t899 isolates clustering apart based on STs. Although *spa* typing has a remarkable predictive power over clonal relationships, predicting genetic relatedness based on *spa* type does not appear appropriate for isolates that have undergone major recombination events, including *spa*

**TABLE 1** Genotypic features of 34 methicillin-resistant *Staphylococcus aureus spa* type t899 isolates<sup>a</sup>

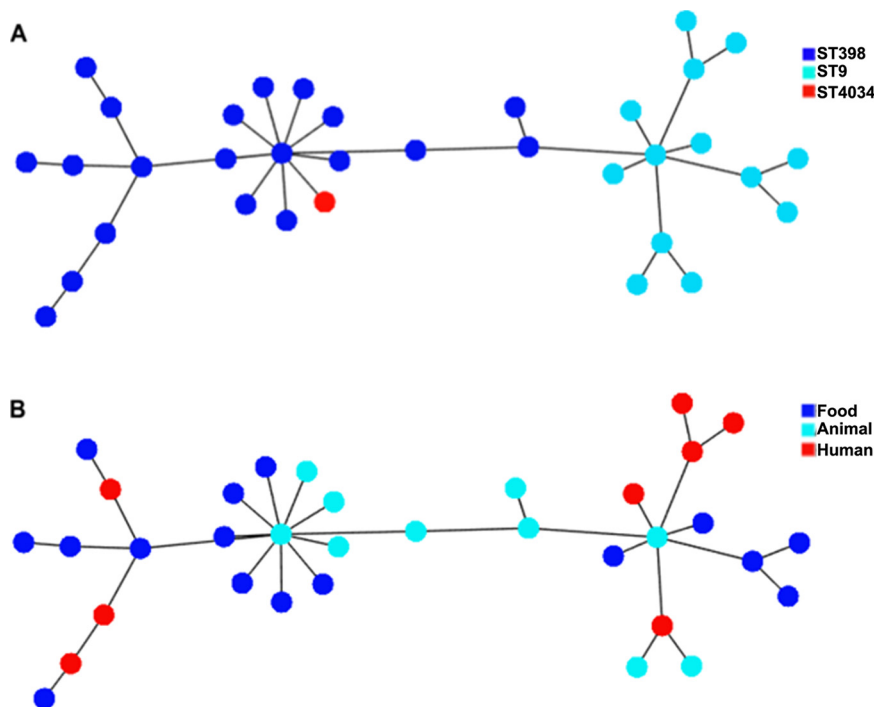
Accession no.	Isolate ID	Yr of isolation	Geographic origin	Isolate source	MLST	SCCmec	Tetracycline resistance gene
SRR1290901	28114	2013	Germany	Human	ST9	IVa(2B)	ND
SRR1290895	28144	2013	Germany	Poultry	ST9	ND	ND
SRR1303238	28194	2010	Netherlands	Human	ST9	IVa(2B)	<i>tet(L)</i>
SRR1290877	28212	—	Germany	Animal	ST9	IVa(2B)	<i>tet(L)</i>
SRR1303551	29908	—	Germany	Poultry	ST9	IVa(2B)	<i>tet(L)</i>
SRR1303423	29933	2010	Netherlands	Human	ST9	IVa(2B)	<i>tet(L)</i>
SRR1303430	30017	2011	Netherlands	Human	ST9	IVa(2B)	ND
ERR594184	30893	2014	Germany	Human	ST9	IVa(2B)	ND
SRR7825591	SAV1035	2017	Poland	Poultry meat	ST9	IVa(2B)	ND
SRR7825590	SAV1149	2017	Germany	Poultry meat	ST9	IVa(2B)	ND
SRR7825587	SAV1150	2017	Germany	Poultry meat	ST9	IVa(2B)	ND
SRR7825588	SAV1158	2017	Germany	Poultry meat	ST9	IVa(2B)	ND
SRR7825595	SAV1228	2017	Czech Republic	Pork	ST9	IVa(2B)	ND
ERR2442746	APHA06	2016	England	Turkey	ST398	IVa(2B)	<i>tet(M)</i>
ERR2562460	PHE3997901	2017	Scotland	Pheasant	ST398	IVa(2B)	<i>tet(M)</i>
SRR1218618	10S00488	2010	Germany	Turkey meat	ST398*	IVa(2B)	<i>tet(M)</i>
SRR1290866	81070	—	Germany	Human	ST398	IVa(2B)	<i>tet(M)</i>
SRR1290867	11S01586	2011	Germany	Camembert	ST398*	IVa(2B)	<i>tet(M)</i>
SRR1290868	12-ST01988	2012	Germany	Turkey meat	ST398*	IVa(2B)	<i>tet(M)</i>
SRR1290875	12S01399	2012	Germany	Turkey meat	ST398*	IVa(2B)	<i>tet(M)</i> , <i>tet(K)</i>
SRR1300909	63-D10	2012	France	Turkey	ST398*	IVa(2B)	<i>tet(M)</i>
SRR1303281	12S00881	2012	Germany	Turkey meat	ST398*	IVa(2B)	<i>tet(M)</i>
SRR1303432	2010-60-7626-19	2010	Denmark	Chicken meat	ST398*	IVa(2B)	<i>tet(M)</i>
SRR1303468	12S01153	2012	Germany	Turkey meat	ST398*	IVa(2B)	<i>tet(M)</i>
SRR1303550	81629	—	Denmark	Human	ST398	IVa(2B)	<i>tet(M)</i>
SRR1303558	10S01493	2010	Germany	Turkey meat	ST398*	IVa(2B)	<i>tet(M)</i>
SRR445027	12152-5	2008	Italy	Pig	ST398*	IVa(2B)	<i>tet(M)</i>
SRR445029	30116	2008	Italy	Pig	ST398*	V(5C2&5)	<i>tet(M)</i>
SRR445030	44523-1	2008	Italy	Pig	ST398*	IVa(2B)	<i>tet(M)</i>
SRR445060	UB08116	2008	France	Pig (dust)	ST398	IVa(2B)	<i>tet(M)</i>
SRR7825589	SAV1146	2017	Germany	Poultry meat	ST398	IVa(2B)	<i>tet(M)</i>
SRR7825592	SAV1109	2017	Poland	Poultry meat	ST398	IVa(2B)	<i>tet(M)</i>
SRR7825593	SAV0154	2013	Czech Republic	Pork	ST4034	IVa(2B)	<i>tet(M)</i>
SRR7825594	SAV0987	2017	Czech Republic	Human	ST398	IVa(2B)	<i>tet(M)</i>

<sup>a</sup>\*, CC398/CC9 hybrid strain; *tet*, tetracycline resistance gene; —, not available; ND, not detected. Isolates with SAV numbers are unique to this study.

gene passages (26–31). This important genomic recombination is not frequent in *S. aureus*, and the major representatives of such events are ST239, ST34, and ST42. The CC9/CC398 hybrid is another important example, giving rise to t899 isolates which largely diverge from their original CC9 genetic backgrounds and which mediate human diseases given their arsenal of virulence factors.

Here, we characterized t899 isolates from different STs using whole-genome-sequencing (WGS)-based approaches, together with epidemiological data, antimicrobial resistance genes, and virulence markers. This analysis revealed the differential occurrence of genes that can be used to further characterize t899 isolates. ST398-t899 isolates harbored the IEC cluster, which is crucial for disrupting the normal function of the human immune system (22, 32–34). Among the 34 t899 isolates tested, all ST398 representatives harbored the *tet(M)* gene, which is either transposon located or chromosomal, while ST9 representatives either were susceptible or carried the plasmid-located *tet(L)* gene. The tetracycline resistance gene *tet(M)* is a common feature of LA-MRSA ST398, while it is absent from MRSA ST9 (35–37). In contrast, ST9 isolates carried staphylococcal enterotoxin (SE) genes, which were not detected in ST398 isolates. This clear discrepancy between the two lineages would be useful to refine the LA-MRSA characterization when only *spa* typing is used and indicates the presence of t899 isolates.

Overall, investigations into *S. aureus* populations using WGS would be useful for future molecular epidemiology studies and for more closely examining the global evolution of *S. aureus* lineages. WGS also helps to assess the performances of classical typing methods by comparison. According to David et al. (38), two genotyping methods



**FIG 1** Minimum spanning tree based on the concatenated core genome of 34 *S. aureus* t899 strains with color annotation based on (A) MLST and (B) matrix. Visualization was realized using PHYLOViZ.

examining distinct genetic loci will not consistently provide identical results in classifying MRSA isolates, mostly because these methods assess genetic differences that can evolve independently. Classification systems often employed for epidemiological research have created competing nomenclatures that are useful for assessing the relatedness of isolates but are unfortunately not always directly comparable. This study emphasizes that *spa* typing is not sufficient to characterize t899-positive LA-MRSA. Accordingly, this study suggests the usefulness of an additional genomic marker to assign t899-positive MRSA isolates to the ST9 or ST398 clone, which may include *tet* (M), *sak*, and/or *seg* genes. Of course, this analysis should be refined when new t899 isolates belonging to other STs are sequenced and characterized.

## MATERIALS AND METHODS

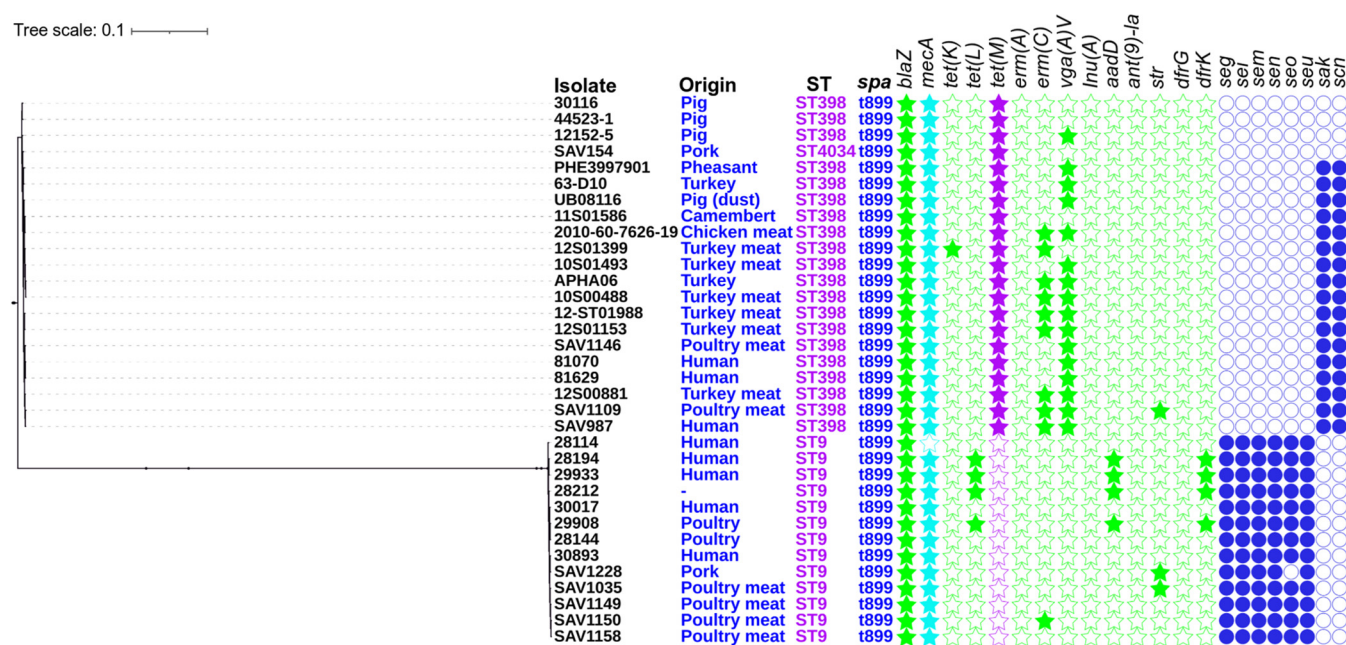
**Bacterial collection.** Thirty-four t899 *S. aureus* isolates were found in the publicly available databases, and their corresponding characteristics (MLST, matrix [human, food, and animal origins], and geographical origin) were recorded. Raw reads were downloaded from NCBI, reads were quality checked with FastQC v.0.65, and low-quality reads were trimmed using Trimmomatic v.0.36.4 (39). Subsequently, contigs were generated using the SPAdes v.3.5.0 algorithm (40), and those whose length exceeded 200 bp were retained in the assembly. In the literature, *spa* type t899 was also found to belong to 15 other SLVs and multilocus variants (MLVs) of ST9 and ST398 (see Table S1 in the supplemental material). These isolates could unfortunately not be included in our analysis because of the absence of associated WGS data.

**cgMLST analyses.** Isolates were subjected to cgMLST analyses. Genome-wide gene-by-gene microbial typing was performed using Ridom SeqSphere+ *S. aureus* cgMLST analysis with default parameters (41). The cgMLST data contain 1,861 coding loci representing the core genome (41). Once an allelic profile was assigned to each genome, a minimum spanning tree was constructed from the concatenated core genome sequences and visualized using the online tool PHYLOViZ. cgMLST loci with no allele calls were ignored in the pairwise comparison during the tree construction. The minimum spanning tree constructed on the basis of cgMLST data illustrates clusters by ST, *spa* type, or matrix (Fig. 1).

**SNP analysis and phylogenetic tree.** A phylogenetic tree was constructed based on single nucleotide polymorphism (SNP) analysis (9, 10, 22). SNPs were identified by mapping reads against the ST398 reference genome (strain S0385; GenBank accession no. [AM990992](https://doi.org/10.1093/nar/21.12.2701)). The maximum-likelihood phylogenetic tree was established in CSI Phylogeny using default settings (42). The phylogenetic tree visualization was realized using iTOL (Interactive Tree of Life) (43).

**Detection of resistance genes and selected virulence markers using WGS data.** The online tools ResFinder v.3.2 (44) and Virulence Finder v. 2.0 (45) from the Center for Genomic Epidemiology web-





**FIG 2** Maximum-likelihood phylogenetic tree based on SNP analyses of 34 *S. aureus* t899 strains obtained by CSI Phylogeny from the Center for Genomic Epidemiology web-based platform. Sequence type (ST), *spa* type (*spa*), isolate origin, the presence of antimicrobial resistance, and virulence markers are indicated. The filled stars and circles indicate confirmed antimicrobial resistance and virulence markers, respectively, as extracted from ResFinder and Virulence Finder. Visualization was realized using iTOL. Isolates with SAV numbers are unique to this study. Genes encoding resistance to beta-lactams (*blaZ*, *mecA*), tetracycline [*tet(K)*, *tet(M)*, *tet(L)*], macrolide-lincosamide-streptogramin B [*erm(A)*, *erm(C)*], pleuromutilin-lincosamide-streptogramin A [*vga(A)V*], lincosamides [*lnu(A)*], aminoglycosides [*aacD*, *ant(9)-Ia*, *str*], and trimethoprim (*dfrK*, *dfrG*) and those encoding staphylococcal enterotoxins (*seg*, *sei*, *sem*, *sen*, *seo*, *seu*), staphylokinase (*sak*), and staphylococcal complement inhibitor (*scn*) are shown.

based platform were used to detect genes encoding potential resistance to antimicrobials and virulence markers, respectively. For a hit to be reported by the two programs, it had to cover at least 60% of the length of the gene sequence in the database with sequence identities of 60% and 90%, respectively. WGS-assembled data were used to perform the analysis.

**Data availability.** The sequence information for isolates SAV1035, SAV1149, SAV1150, SAV1158, and SAV1228 has been deposited in the SRA database under study accession number [SRP161670](https://www.ncbi.nlm.nih.gov/sra/ERP161670). Individual accession numbers are listed in Table 1.

## SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

**SUPPLEMENTAL FILE 1**, PDF file, 0.1 MB.

## ACKNOWLEDGMENTS

We thank the service Transversal Activities in Applied Genomics from Sciensano for the paired-end sequencing reactions and for the development and maintenance of the in-house instance of the Galaxy workflow management system and Andrew D. Miller for his help in proofreading the manuscript. We thank Mirko Rossi for his valuable input in the course of designing this study and Beatriz Guerra and Antonio Rinaldi for cgMLST analysis.

This study was supported by the Ministry of Education, Youth and Sports, project no. CZ.1.05/2.1.00/19.0385. Funding sources did not affect the design of this study, data collection, data analysis, decisions on publication, or preparation of the manuscript.

## REFERENCES

- Armand-Lefevre L, Ruimy R, Andremont A. 2005. Clonal comparison of *Staphylococcus* from healthy pig farmers, human controls, and pigs. *Emerg Infect Dis* 11:711–714. <https://doi.org/10.3201/eid1105.040866>.
- Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M. 2005. Methicillin-resistant *Staphylococcus aureus* in pig farming. *Emerg Infect Dis* 11:1965–1966. <https://doi.org/10.3201/eid1112.050428>.
- Butaye P, Argudin MA, Smith TC. 2016. Livestock-associated MRSA and its current evolution. *Curr Clin Micro Rpt* 3:19–31. <https://doi.org/10.1007/s40588-016-0031-9>.
- Ye X, Wang X, Fan Y, Peng Y, Li L, Li S, Huang J, Yao Z, Chen S. 2016. Genotypic and phenotypic markers of livestock-associated methicillin-resistant *Staphylococcus aureus* CC9 in humans. *Appl Environ Microbiol* 82:3892–3899. <https://doi.org/10.1128/AEM.00091-16>.
- Cui S, Li J, Hu C, Jin S, Li F, Guo Y, Ran L, Ma Y. 2009. Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from swine and

- workers in China. *J Antimicrob Chemother* 64:680–683. <https://doi.org/10.1093/jac/dkp275>.
6. Frana TS, Beahm AR, Hanson BM, Kinyon JM, Layman LL, Karriker LA, Ramirez A, Smith TC. 2013. Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from pork farms and visiting veterinary students. *PLoS One* 8:e53738. <https://doi.org/10.1371/journal.pone.0053738>.
  7. Hau SJ, Allué-Guardia A, Rusconi B, Haan JS, Davies PR, Frana TS, Eppinger M, Nicholson TL. 2018. Single nucleotide polymorphism analysis indicates genetic distinction and reduced diversity of swine-associated methicillin resistant *Staphylococcus aureus*(MRSA) ST5 isolates compared to clinical MRSA ST5 isolates. *Front Microbiol* 9:2078. <https://doi.org/10.3389/fmicb.2018.02078>.
  8. Guardabassi L, O'Donoghue M, Moodley A, Ho J, Boost M. 2009. Novel lineage of methicillin-resistant *Staphylococcus aureus*, Hong Kong. *Emerg Infect Dis* 15:1998–2000. <https://doi.org/10.3201/eid1512.090378>.
  9. Larsen J, Stegger M, Andersen PS, Petersen A, Larsen AR, Westh H, Agersø Y, Fetsch A, Kraushaar B, Käsbohrer A, Feßler AT, Schwarz S, Cuny C, Witte W, Butaye P, Denis O, Haenni M, Madec JY, Jouy E, Laurent F, Battisti A, Franco A, Alba P, Mammina C, Pantosti A, Monaco M, Wagenaar JA, De Boer E, Van Duikeren E, Heck M, Domínguez L, Torres C, Zarazaga M, Price LB, Skov RL. 2016. Evidence for human adaptation and foodborne transmission of livestock-associated methicillin-resistant *Staphylococcus aureus*. *Clin Infect Dis* 63:1349–1352. <https://doi.org/10.1093/cid/ciw532>.
  10. Sharma M, AbuOun M, Nunez-Garcia J, Rogers J, Welchman D, Teale C, Anjum MF, Kearns AM, Pichon B, Foster G, Robb A, McMillan M. 2018. MRSA *spa* type t899 from food animals in the UK. *Vet Rec* 182:697–698. <https://doi.org/10.1136/vr.k2576>.
  11. Pan A, Battisti A, Zoncada A, Bernieri F, Boldini M, Franco A, Giorgi M, Iurescia M, Lorenzotti S, Martinotti M, Monaci M, Pantosti A. 2009. Community-acquired methicillin-resistant *Staphylococcus aureus* ST398 infection, Italy. *Emerg Infect Dis* 15:845–847. <https://doi.org/10.3201/eid1505.081417>.
  12. Monaco M, Pedroni P, Sanchini A, Bonomini A, Indelicato A, Pantosti A. 2013. Livestock-associated methicillin-resistant *Staphylococcus aureus* responsible for human colonization and infection in an area of Italy with high density of pig farming. *BMC Infect Dis* 13:258. <https://doi.org/10.1186/1471-2334-13-258>.
  13. Larsen J, Petersen A, Larsen AR, Sieber RN, Stegger M, Koch A, Aarestrup FM, Price LB, Skov RL, Johansen HK, Westh H, Pedersen M, Jensen US, Jensen MLS, Chen M, Strøbæk S, Østergaard C, Lomborg S, Ellermann-Eriksen S, Ripadál P, Danish MRSA Study Group. 2017. Emergence of livestock-associated methicillin-resistant *Staphylococcus aureus* bloodstream infections in Denmark. *Clin Infect Dis* 65:1072–1076. <https://doi.org/10.1093/cid/cix504>.
  14. Köck R, Apel J, Gerth C, Gundlach S, Lambrecht C, Josef TS. 2010. Prevalence of MRSA CC398 in pig holdings and human hospital patients in North Rhine-Westphalia, Germany. *Methods* 3:2010.
  15. Köck R, Schaumburg F, Mellmann A, Köksal M, Jurke A, Becker K, Friedrich AW. 2013. Livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA) as causes of human infection and colonization in Germany. *PLoS One* 8:e55040. <https://doi.org/10.1371/journal.pone.0055040>.
  16. Schulz J, Boklund A, Toft N, Halasa T. 2018. Drivers for livestock-associated methicillin-resistant *Staphylococcus aureus* spread among Danish pig herds—a simulation study. *Sci Rep* 8:1–11. <https://doi.org/10.1038/s41598-018-34951-1>.
  17. Tegegne HA, Koláčková I, Karpišková R. 2017. Diversity of livestock associated methicillin-resistant *Staphylococcus aureus*. *Asian Pac J Trop Med* 10:929–931. <https://doi.org/10.1016/j.apjtm.2017.08.013>.
  18. Pirolo M, Giuffrè A, Visaggio D, Gherardi M, Pavia G, Samele P, Ciambone L, Di Natale R, Spatarì G, Casaliniuofo F, Visca P. 2019. Prevalence, molecular epidemiology, and antimicrobial resistance of methicillin-resistant *Staphylococcus aureus* from swine in southern Italy. *BMC Microbiol* 19:1–12. <https://doi.org/10.1186/s12866-019-1422-x>.
  19. Battisti A, Franco A, Merialdi G, Hasman H, Iurescia M, Lorenzetti R, Feltrin F, Zini M, Aarestrup FM. 2010. Heterogeneity among methicillin-resistant *Staphylococcus aureus* from Italian pig finishing holdings. *Vet Microbiol* 142:361–366. <https://doi.org/10.1016/j.vetmic.2009.10.008>.
  20. Monecke S, Coombs G, Shore AC, Coleman DC, Akpaka P, Borg M, Chow H, Ip M, Jatzwauk L, Jonas D, Kadlec K, Kearns A, Laurent F, O'Brien FG, Pearson J, Ruppelt A, Schwarz S, Scicluna E, Slickers P, Tan H-L, Weber S, Ehrlich R. 2011. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*. *PLoS One* 6:e17936. <https://doi.org/10.1371/journal.pone.0017936>.
  21. Stone K. 2017. Risk assessment on methicillin-resistant *Staphylococcus aureus* (MRSA), with a focus on livestock-associated MRSA, in the UK food chain. Food Standards Agency, London, United Kingdom.
  22. Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, Pearson T, Waters AE, Foster JT, Schupp J, Gillette J, Driebe E, Liu CM, Springer B, Zdovc I, Battisti A, Franco A, Z<sup>2</sup>Mudzki J, Schwarz S, Butaye P, Jouy E, Pomba C, Porrero MC, Ruimy R, Smith TC, Robinson DA, Weese JS, Arriola CS, Yu F, Laurent F, Keim P, Skov R, Aarestrup FM. 2012. *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. *mBio* 3:e00305-11. <https://doi.org/10.1128/mBio.00305-11>.
  23. Yamamoto T, Takano T, Higuchi W, Iwao Y, Singur O, Reva I, Otsuka Y, Nakayashiki T, Mori H, Reva G, Kuznetsov V, Potapov V. 2012. Comparative genomics and drug resistance of a geographic variant of ST239 methicillin-resistant *Staphylococcus aureus* emerged in Russia. *PLoS One* 7:e29187. <https://doi.org/10.1371/journal.pone.0029187>.
  24. Thomas JC, Godfrey PA, Feldgarden M, Robinson DA. 2012. Draft genome sequences of *Staphylococcus aureus* sequence type 34 (ST34) and ST42 hybrids. *J Bacteriol* 194:2740–2741. <https://doi.org/10.1128/JB.00248-12>.
  25. Bartels MD, Worning P, Andersen LP, Bes M, Enger H, Ås CG, Hansen TA, Holzkecht BJ, Larssen KW, Laurent F, Mäkitalo B, Pichon B, Svartström O, Westh H. 2020. Repeated introduction and spread of the MRSA clone t304/ST6 in northern Europe. *Clin Microbiol Infect* <https://doi.org/10.1016/j.cmi.2020.05.004>.
  26. Asadollahi P, Farahani NN, Khoramrooz SS, van Belkum A, Asadollahi K, Dadashi M, Darban-Sarokhalil D. 2018. Distribution of the most prevalent *spa* types among clinical isolates of methicillin-resistant and -susceptible *Staphylococcus aureus* around the world: a review. *Front Microbiol* 9:163. <https://doi.org/10.3389/fmicb.2018.00163>.
  27. Bosch T, Pluister GN, van Luit M, Landman F, van Santen-Verheul M, Schot C, Witteveen S, van der Zwaluw K, Heck MEOC, Schouls LM. 2015. Multiple-locus variable number tandem repeat analysis is superior to *spa* typing and sufficient to characterize MRSA for surveillance purposes. *Future Microbiol* 10:1155–1162. <https://doi.org/10.2217/fmb.15.35>.
  28. Darban-Sarokhalil D, Khoramrooz SS, Marashifard M, Malek Hosseini SAA, Parhizgari N, Yazdanpanah M, Gharibpour F, Mirzaii M, Sharifi B, Haeili M. 2016. Molecular characterization of *Staphylococcus aureus* isolates from southwest of Iran using *spa* and SCCmec typing methods. *Microb Pathog* 98:88–92. <https://doi.org/10.1016/j.micpath.2016.07.003>.
  29. Koreen L, Ramaswamy SV, Graviss EA, Naidich S, Musser JM, Kreiswirth BN. 2004. *spa* typing method for discriminating among *Staphylococcus aureus* isolates: implications for use of a single marker to detect genetic micro- and macrovariation. *J Clin Microbiol* 42:792–799. <https://doi.org/10.1128/jcm.42.2.792-799.2004>.
  30. O'Hara FP, Suaya JA, Ray GT, Baxter R, Brown ML, Mera RM, Close NM, Thomas E, Amrine-Madsen H. 2016. *spa* typing and multilocus sequence typing show comparable performance in a macroepidemiologic study of *Staphylococcus aureus* in the United States. *Microb Drug Resist* 22:88–96. <https://doi.org/10.1089/mdr.2014.0238>.
  31. Ruppitsch W, Indra A, Stöger A, Mayer B, Stadlbauer S, Wewalka G, Allerberger F. 2006. Classifying *spa* types in complexes improves interpretation of typing results for methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 44:2442–2448. <https://doi.org/10.1128/JCM.00113-06>.
  32. Cuny C, Wieler L, Witte W. 2015. Livestock-associated MRSA: the impact on humans. *Antibiotics (Basel)* 4:521–543. <https://doi.org/10.3390/antibiotics4040521>.
  33. McCarthy AJ, Witney AA, Gould KA, Moodley A, Guardabassi L, Voss A, Denis O, Broens EM, Hinds J, Lindsay JA. 2011. The distribution of mobile genetic elements (MGEs) in MRSA CC398 is associated with both host and country. *Genome Biol Evol* 3:1164–1174. <https://doi.org/10.1093/gbe/evr092>.
  34. Hau SJ, Sun J, Davies PR, Frana TS, Nicholson TL. 2015. Comparative prevalence of immune evasion complex genes associated with  $\beta$ -hemolysin converting bacteriophages in MRSA ST5 isolates from swine, swine facilities, humans with swine contact, and humans with no swine contact. *PLoS One* 10:e0142832. <https://doi.org/10.1371/journal.pone.0142832>.
  35. Gordon NC, Price JR, Cole K, Everitt R, Morgan M, Finney J, Kearns AM, Pichon B, Young B, Wilson DJ, Llewellyn MJ, Paul J, Peto TEA, Crook DW, Walker AS, Golubchik T. 2014. Prediction of *Staphylococcus aureus* antimicrobial resistance by whole-genome sequencing. *J Clin Microbiol* 52:1182–1191. <https://doi.org/10.1128/JCM.03117-13>.
  36. Liu B, Pop M. 2009. ARDB—antibiotic resistance genes database. *Nucleic Acids Res* 37:443–447.
  37. Rossney AS, Shore AC, Morgan PM, Fitzgibbon MM, O'Connell B, Coleman DC. 2007. The emergence and importation of diverse genotypes of methicillin-resistant *Staphylococcus aureus* (MRSA) harboring the panton-

- valentine leukocidin gene (*pvl*) reveal that *pvl* is a poor marker for community-acquired MRSA strains in Ireland. *J Clin Microbiol* 45:2554–2563. <https://doi.org/10.1128/JCM.00245-07>.
38. David MZ, Taylor A, Lynfield R, Boxrud DJ, Short G, Zychowski D, Boyle-Vavra S, Daum RS. 2013. Comparing pulsed-field gel electrophoresis with multilocus sequence typing, *spa* typing, staphylococcal cassette chromosome *mec* (*SCCmec*) typing, and PCR for Panton-Valentine leukocidin, *arcA*, and *opp3* in methicillin-resistant *Staphylococcus aureus* isolates. *J Clin Microbiol* 51:814–819. <https://doi.org/10.1128/JCM.02429-12>.
  39. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
  40. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
  41. Leopold SR, Goering RV, Witten A, Harmsen D, Mellmann A. 2014. Bacterial whole-genome sequencing revisited: portable, scalable, and standardized analysis for typing and detection of virulence and antibiotic resistance genes. *J Clin Microbiol* 52:2365–2370. <https://doi.org/10.1128/JCM.00262-14>.
  42. Kaas RS, Leekitcharoenphon P, Aarestrup FM, Lund O. 2014. Solving the problem of comparing whole bacterial genomes across different sequencing platforms. *PLoS One* 9:e104984. <https://doi.org/10.1371/journal.pone.0104984>.
  43. Letunic I, Bork P. 2019. Interactive Tree of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res* 47:W256–W259. <https://doi.org/10.1093/nar/gkz239>.
  44. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* 67:2640–2644. <https://doi.org/10.1093/jac/dks261>.
  45. Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, Aarestrup FM. 2014. Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic *Escherichia coli*. *J Clin Microbiol* 52:1501–1510. <https://doi.org/10.1128/JCM.03617-13>.