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Author: Xiaomei Yan Zongwei Li Monika A. Chlebowicz Xiaoxia Tao Ming Ni Yuan Hu Zhen Li Hajo Grundmann Susan Murray Ben Pascoe Samuel K. Sheppard Xiaochen Bo Jan Maarten van Dijl Pengcheng Du Minli Zhang Yuanhai You Xiaojie Yu Fanliang Meng Shengqi Wang Jianzhong Zhang

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Genetic features of livestock-associated *Staphylococcus aureus* ST9 isolates from Chinese pigs that carry the *lsa*(E) gene for quinupristin/dalfopristin resistance

Running title: Genomes of *lsa*(E) carrying strains

Xiaomei Yan[a,d*], Zongwei Li[b*], Monika A. Chlebowicz[c], Xiaoxia Tao[a,d], Ming Ni[b], Yuan Hu[a], Zhen Li[b], Hajo Grundmann[h], Susan Murray[f], Ben Pascoe[f,g], Samuel K. Sheppard[f,g], Xiaochen Bo[b], Jan Maarten van Dijl[c], Pengcheng Du[a,d], Minli Zhang[b], Yuanhai You[a,d], Xiaojie Yu[e], Fanliang Meng[a,d], Shengqi Wang[b#], and Jianzhong Zhang[a,d#]

[a] State Key Laboratory of Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China

[b] Beijing Institution of Radiation Medicine, Beijing, China

[c] Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, The Netherlands

[d] Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Hangzhou, China

[e] Heilongjiang provincial Centre for Disease Control and Prevention, Harbin, China

[f] Swansea University Medical School, Institute of Life Sciences, Swansea University, Singleton Park, Swansea, UK

[g] The Milner Centre for Evolution, Department of Biology and Biochemistry, University of Bath, Bath, United Kingdom

[h] Department of Infection Prevention and Hospital Hygiene, University Medical Centre Freiburg, Freiburg, Germany

* These authors contributed equally to this work

#Corresponding authors:

Tel: +86-10-58900707; Fax: +86-10-58900790;

Email: zhangjianzhong@icdc.cn

Address: 155 Changbai Road, Changping District, Beijing, China 102206

Tel: +86-10-68210077; Fax: +86-10-66932211;

Email: sqwang@bmi.ac.cn

Address: 27 Taiping Road, Haidian District, Beijing 100850, China

Abstract

Whole-genome sequencing (WGS) was used to investigate the genetic features of the recently identified *lsa*(E) gene in porcine *S. aureus* ST9 isolates. Three quinupristin/ dalfopristin-resistant isolates harboring the *lsa*(E) gene (two MRSA and one MSSA) were sequenced. Phylogenetic analysis of 184 *S. aureus* genomes showed that ST9 porcine isolates belong to a distinct sequence cluster. Further analysis showed that all isolates were deficient in the recently described type IV restriction-modification system and SCC*mec* type XII was identified in the two MRSA isolates, which included a rare class C2 *mec* gene complex. A 24kb ΨSCC fragment was found in the MRSA and MSSA isolates sharing 99% nucleotide sequence homology with the ΨSCCJCSC6690 (O-2) element of a ST9 MRSA isolate from Thailand (accession number AB705453). Comparison of these ST9 isolates with 181 publically available *S. aureus* genomes identified 24 genes present in all (100%) ST9 isolates, that were absent from the most closely related human isolate. Our analysis suggests that the sequenced quinupristin/ dalfopristin-resistant ST9 lineage represent a reservoir of mobile genetic elements associated with resistance and virulence features.

Key words: *Staphylococcus aureus*; Quinupristin/dalfopristin; Resistance; Livestock-associated; ST9; ABC transporters

Introduction

Staphylococcus aureus is an opportunistic pathogen that can rapidly develop resistance to antimicrobial agents (Pantosti et al., 2007). Methicillin-resistant *S. aureus* (MRSA) isolates are common pathogens in hospitals world-wide (Edelsberg et al., 2014; Xiao et al., 2011). Antibiotic resistance in *S. aureus* can emerge through point mutations or horizontal gene transfer of mobile genetic elements (MGEs). MGEs such as plasmids, transposons or insertion (IS) elements can occasionally originate from other bacterial species (Ferrero et al., 1995; Gao et al., 2013; Tsiodras et al., 2001; Weigel et al., 2003, 2007).

Genetic exchange of genes coding antibiotic resistance has been reported between enterococci and *S. aureus* (Weigel et al., 2003, 2007). The *vanA* gene (infers resistance to vancomycin) located on a Tn*1546*-containing plasmid, *tetL* (tetracycline resistance), *dfrK* (trimethoprim resistance) (Lopez et al., 2012) or the multi-resistance gene *cfr* (Liu et al., 2012) are believed to have been acquired by *S. aureus* from enterococci. Recently, a gene encoding an ABC transporter *lsa*(E), which confers resistance to streptogramin A, has been identified in *S. aureus* and may be imported from *Enterococcus* sp. (Wendlandt et al., 2013).

Quinupristin and dalfopristin (collectively termed QDA) belong to the streptogramin A and B antibiotic groups. These structurally distinct cyclic

peptide antibiotics act synergistically on the bacterial 50S ribosomal subunit inhibiting protein synthesis (Johnston et al., 2002) . Virginiamycin belongs to the same antibiotic group as QDA and is widely used as an animal growth promoter in poultry, cattle and swine. In *S. aureus,* resistance to streptogramin B does not confer resistance to QDA while resistance to streptogramin A does (Hancock, 2005). Despite QDA not been marketed in China, virginiamycin is widely used agriculturally. Resistance to streptogramin A-type antibiotics can be caused by different mechanisms, such as acetyltransferase Vat (Allignet et al., 1993, 1998), the ABC transporters Vga (Allignet et al., 1992; Kadlec and Schwarz, 2009; Schwendener and Perreten, 2011) and Lsa (Wendlandt et al., 2013), and the methyltransferase Cfr (Long et al., 2006).

So far, the *lsa*(E) gene has been reported from *S. aureus* of multi locus sequence types ST398, ST125 and ST9 of human (Wendlandt et al., 2013, 2014), pig (Li et al., 2013;Wendlandt et al., 2014), diary cow (Silva et al., 2014b; Wendlandt et al, 2015) and poultry origin (Wendlandt et al., 2014), as well as coagulase-negative staphylococci of dairy cows (Silva et al., 2014a , Wendlandt et al, 2015), group B Streptococcus (Montilla et al, 2014; Douarre et al., 2015), Erysipelothrix rhusiopathiae (Zhang et al.,2015) and enterococci of human and swine origin (Li et al, 2014). Previously we have demonstrated that 98% (44/45) of the QDA-resistant *S. aureus* isolates sampled from slaughter pigs in northeastern China harbored $lsa(E)$, which was also described recently in other regions of China (Li et al., 2013; Li et al., 2014; Yan et al., 2014; Zhang et al., 2015). The $lsa(E)$ gene has to date only been found in isolates belonging to sequence type (ST-) 9 (both MRSA and MSSA) in China. A defect of the DNA restriction-modification system in ST9 isolates may facilitate horizontal gene transfer of foreign DNA, as reported for ST398 (Schijffelen et al., 2010). In this study we investigate the genetic features of $lsa(E)$-positive porcine *S. aureus* ST9 isolates by whole-genome sequencing

(WGS).

Materials and methods

Bacterial isolates, growth conditions and genomic DNA isolation

Three isolates were randomly selected from the 44 QDA-resistant isolates harboring the *lsa*(E) gene strains for genome sequencing. Two of these isolates were MRSA and one was a methicillin-sensitive *S. aureus* (MSSA; Table 1). All three isolates were collected from nasal swabs from healthy pigs in Harbin city, Heilongjiang Province, in northeastern China. All isolates belonged to ST9 and had the *spa* type t899. Isolates were grown on tryptic soy agar (TSA) with 5% sheep blood at 37°C. Genomic DNA of each isolate was prepared using the QIAamp DNA Mini Kit (QIAGEN 51306, GmbH, Hilden, Germany) according to the manufacturer's protocol with an additional cell lysis step involving incubation with 50μg/ml lysostaphin (final concentration) at 37°C for 1 h.

Library preparation

Genomic DNA (gDNA) (1μg per sample) was fragmented with a Covaris S220 focused-ultrasonicator (Covaris, part # SE-501-1001) under the

following conditions: duty factor (10%), peak incident power (175), cycle per burst (200), duration (40 seconds), mode frequency sweeping (frequency sweeping) and temperature (5.5° to 6°C). Libraries were prepared using the Truseq DNA sample preparation kit (FC-121-2001, Illumina) according to the TruSeq DNA sample preparation guide (Part # 15026486 Rev. C, Illumina).

Genome sequencing, assembly and annotation

Genome sequencing was performed using the Illumina MiSeq sequencing system (Illumina, San Diego, CA 92122 USA) according to the MiSeq system user guide. *De novo* assemblies were performed using SOAPdenovo v1.05 (Beijing Genomics Institute at Shenzhen, Shenzhen 518083, China) at an optimal hash length of 107, 107 and 109 for the A69, A71 and A187 strains, respectively. The published genome sequence of *S. aureus* N315 (Accession: NC_002745) was used as the reference genome. Contigs of each strain were resorted according to the N315 sequence by MAUVE (Darling et al., 2010). All three genome assemblies in this paper have been deposited at DDBJ/EMBL/GenBank under the accession numbers JJOP00000000, JJOO00000000 and JJON00000000. The version described here is the first version JJOP01000000, JJOO01000000 and JJON01000000 (to submit the results to NCBI, scaffolds that contain more than 10 continuous Ns were split into smaller contigs and the contigs less than 200bp were filtered out). Pairwise genomic comparisons were generated by blast and analyzed using the Artemis Comparison Tool (ACT) (http://www.sanger.ac.uk/resources/software/artemis/) (Carver et al., 2005).

Generated contigs where annotated using the Rapid Annotations Subsystems Technology (RAST) (Aziz et al., 2008) and Artemis. tRNA- and rRNA-encoding genes were searched by tRNAscan-SE (Lagesen et al., 2007) and RNAmmer, separately (Schattner et al., 2005). Gaps were amplified by PCR using Takara LA Taq polymerase, and the resulting PCR products were sequenced by primer walking (Table S1).

Phylogenetic analysis

Isolate genomes were archived in a web-accessible database that supports functionality for identifying the gene presence and allelic variation by comparison to a reference locus list (Jolley and Maiden, 2010; Sheppard et al., 2012; Meric et al., 2014). This list comprised 2,593 locus designations from the annotated genome of *S. aureus* strain N315 (Genbank accession number: NC_002745) was used as a reference genome. These reference loci were identified in our 3 sequenced genomes and 181 published genomes using BLAST (Table S2). Loci were recorded as present if the sequence had 70% nucleotide identity over 50% of the gene length.

Genes were aligned individually for all 184 genomes, using MUSCLE (Edgar, 2004), and concatenated into a single multi-FASTA alignment file for each isolate. Maximum Likelihood trees were produced using FastTree2 (Price et al., 2010), which allows the reconstruction of branch lengths greater than 0.0000005, corresponding to a minimum branch length of 1 substitution for every 2,000,000 base pairs (1000 times higher than the

default FastTree parameters). The tree was created using a total alignment length of 2,402,099 bp.

Isolation of plasmid DNA

Plasmid DNA of the three isolates was extracted using the Qiagen plasmid extraction midi kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions with the following modification: after re-suspending the bacterial pellet in buffer P1, lysostaphin was added to a final concentration of 0.02mg/L and the mixture was incubated for 1 h at 37ºC before adding buffer P2. To estimate plasmid sizes, digestion using the restriction enzymes *Hin*dIII and *Eco*RI was performed separately on each isolated plasmid.

Results

Genomic characteristics of sequenced ST9 isolates

In this study, two MRSA isolates (A69, A71) and one MSSA isolate (A187) have been sequenced and analyzed. Genome sizes of isolates A69, A71 and A187 were shown to consist of 2,844,421 bp, 2,849,873 bp and 2,786,770 bp, respectively and each isolate was found to contain a single plasmid (3,027 bp, 2,581 bp and 2,990 bp, respectively, Table 2).

Genomic comparisons of the MRSA isolates with the MSSA isolate revealed that the MSSA isolate lacks a prophage (ΦSa2int), a pathogenicity island (SaPI6Δa), and part of SCC*mec* complex (Table 2). Furthermore, the chromosomes of isolates A69, A71 and A187 show approximately 92.66%, 92.67% and 92.49% sequence identity with that of *S. aureus* N315. The major differences observed concern the presence or absence of mobile genetic elements (MGEs). MGEs identified in all three isolates are: the genomic islands α and β, encoding enterotoxins; SaPIbov4-like pathogenicity islands (*vwb, scn, aadE*); the transposons Tn552 (*blaZ*) , Tn552-like (*blaZ*), Tn558 (*fexA*); a transposon-like MGE carrying the *lsa*(E)*, lnu*(B)*, aadE,* and *tetL* genes (Table 3).

Quinupristin / dalfopristin resistant isolates carry lsa(E)

Genetically identical *lsa*(E) gene clusters were observed in all three genomes. The *lsa*(E) gene was embedded in a 12.2 kb MGE flanked by two IS257 sequences. Comparative analysis revealed that the *lsa*(E) gene cluster shows 99% nucleotide sequence identity with genetic structures (accession number JQ861959) found in one MRSA isolate that belongs to ST398 and two human MSSA isolates with ST9 from Spain. Furthermore, the identified MGE with the *lsa*(E) gene cluster shows similarity to plasmid pEF418 of *E. faecalis* (accession number AF408195), plasmid pXD4 from *E. faecium* (accession number KF421157) and plasmid pV7037 from swine MRSA originating from China (accession number JX560992) (Fig. 1). The MGE carrying *lsa*(E) is positioned adjacent to another transposon with IS257 containing the *tetL* tetracycline resistance gene upstream, which is 99% identical to a gene cluster found on *B. cereus* plasmid pBC16 (accession number AAA84922), the *E. faecium* plasmid pM7M2 (accession number JF800907), and the *S. aureus* SA7037 plasmid pV7037 (accession number HF586889). The *lsa*(E) gene and homologues are found only in the ST9 isolates when compared to 184 published *S. aureus* isolates (Table S3).

Both MRSA ST-9 isolates contain SCCmec type XII

The identified SCC*mec* is 48.575 kb in length and contains 45 predicted open reading frames (Table S4). The G+C content of this SCC*mec* is 31.98%, which is slightly lower than the overall G+C content of 32.8% of the remaining genome. The SCC*mec* contains three integration site sequences (ISS) consisting of direct repeats (DR) that divide this MGE into two regions. The first DR is located within *orfX* and is identical to

DR2 present in SCC*mec* JCSC6690 (O-2). The second DR, unique for this SCC*mec*, delimits region one and two, while the third DR is located at the end of this mobile element and is identical to DR3 on SCC*mec* JCSC6690 (O-2) (Fig. 2). Region I, downstream of *orfX*, is 24.293kb in size and contains a pseudoSCC element with a truncated *ccrA1* gene. Overall the structure of this region shares 99% of nucleotide sequence identity with ΨSCCJCSC6690 (O-2) located on the SCC*mec* element of ST9 MRSA strain JCSC6690 (accession number AB705453). Region II is 24.282 kb in size and contains the *ccrC* gene cluster and a *mec* gene complex. The *ccrC* allotype displayed 68%-70% DNA sequence similarity with known staphylococcal *ccrC1* alleles (Table S5) and 98% amino acid identity with a phage transcriptional activator of *S. epidermidis*. Additional hypothetical genes in the neighborhood of *ccrC* are highly similar to proteins from *S. hominis*. Further, within the described region a 13.8 kb typical class C2 *mec* gene complex is located, after which a restriction modification-like structure is present. This new restriction modification-like structure consists of two novel proteins that share low overall similarity (45% and 31%) in amino acid sequence with a known methyltransferase (accession number WP_005807159.1) and a type III restriction enzyme (accession number WP_000891153.1), respectively.

Potential remnant SCC*mec* elements, including the DR2 and DR3 direct repeats, were present in the genome of the MSSA isolate. This potential SCC*mec* remnant of 24.7 kb comprises 23 predicted genes that are identical to equivalent genes present within the respective regions of the SCC*mec* elements of both sequenced MRSA isolates (Fig. 2).

All three sequenced ST9 isolates carry a SaPIbov4-like pathogenicity island

A single prophage, Sa2int was identified in both MRSA genomes, which is similar to the prophage previously found in the sequenced *S. aureus* ST398 genome (SO385). The prophage Sa2int of 44 kb is integrated within the gene coding for 6-phospho-beta-galactosidase (lacG) and does not carry known virulence determinants (Table 3). All three sequenced ST9 isolates carry a SaPIbov4-like pathogenicity island integrated downstream of the *guaA* gene for GMP synthase. In general, this mobile element is very similar in structure to the previously reported SaPIbov4 (accession number HM211303.1). However, clear differences are detectable at the 3' terminal part. The identified SaPI carries the genes for an animal-associated staphylococcal complement inhibitor (*scn*) and a von Willebrand factor binding protein (*vwb*) that both have an inverted orientation compared to SaPIbov4 (Fig. S1). Additionally, this new SaPIbov4-like element encodes an aminoglycoside 6-adenylyltransferase (*aadE*) in the 3' terminal.

MGEs from other species

In addition to the *lsa*(E) gene cluster and rare SCC*mec* structure, two transposon-like elements were found in all three sequenced genomes. The first transposon-like element is inserted into the gene for the L-lactate permease (accession number EHM74991.1). It carries six genes including

the gene for a Tn552 transposase (Table 4). This novel gene structure is physically linked with a Tn552 transposon carrying *blaZ*. The second transposon-like element is inserted downstream the gene for a hypothetical protein belonging to an enterotoxin homology group (accession number WP_001792564.1), and it carries four genes not found in *S. aureus* before (Table 4).

Phylogenetic relationship of ST9 isolates with published S. aureus isolates

The phylogenetic relationships between the sequenced genomes of A69, A71, A187 and 184 publicly available *S. aureus* genomes on NCBI were compared. The Asian ST9 livestock-associated *S. aureus* isolates are genetically distinct from the livestock-associated CC398 isolates that predominate in Europe (Fig. 3). When we compare the ST9 pig isolates to the closest related human strain based on the tree (ID: 147), we find 24 porcine-associated genes (present 100% in pig iosolates, but not in human isolate id: 147; Table S6).

Restriction modification system (RM) of ST9

To better understand the mechanisms of *lsa*(E) gene transfer, the RM systems of the three sequenced ST9 isolates were analyzed. The intact type I RM system was found in all three strains sharing 100% nucleotide identity with one copy of the *hsdR* gene, which encodes the restriction

subunit, and two different copies of *hsdMS*, which encode proteins for recognition and modification of specific sequences. *HsdR* and two copies of *hsdM* were conserved with only one or two mutations compared to published sequences of other *S. aureus* isolates. All three strains possessed an amino acid substitution in the *hsdR* gene from arginine to lysine (R873K) when compared with *hsdR* of the N315 and 21334 strains (Fig. S2). The genes for the methyltransferase HsdM and the specificity subunit HsdS are located within genomic islands, νSaα and νSaβ, respectively. There is 100% sequence similarity between *hsdM1* located on νSaα and the *hsdM* gene of strain 21334. The second copy of *hsdM2* located on νSaβ contains one amino acid substitution (S60A) compared to the *hsdM* gene of the 21334 strain (Fig. S2). The lineage-specific *hsdS1* gene located within γSaα contains a specific region (encoding amino acid residues 24-171) that seems to be unique for HsdS1 as no similar sequence has been found in the NCBI database. The *hsdS2* located in γSaβ encodes a protein with 99% amino acid sequence similarity to the HsdS2 proteins of strains 21334, MO483, Co-08 and KT/Y21 (Fig. S2).

Genes for the intact type II RM-*Sau*3AI were found on the chromosomes of the sequenced ST9 isolates. This RM system consists of two enzymes, a restriction enzyme and the cognate modification enzyme. All isolates also possess a recently described type IV RM system (originally referred to as a type III system) that appears defective due to the loss of the corresponding *hsdR* gene, as confirmed by PCR (Fig. S3). The 41 *lsaE* carrying strains were all deficient of *hsdR* according to PCR detection.

Discussion

In this study we describe the genetic features of 3 whole genome sequenced *S. aureus* isolates carrying the *lsa*(E) gene, which is associated with quinupristin/ dalfopristin resistance. In South-East Asia the ST9 clonal complex is the most widely sampled among farmed pigs (Yan et al., 2014). In our study, ST9 is genetically distinct from other livestock-associated staphylococcal lineages, including CC398 which has successfully spread among pigs in Europe (Lekkerkerk et al., 2015; Molla et al., 2012). The genetic distinctiveness of ST9 is consistent with emergence following an independent zoonotic event, and has a different evolutionary background to other typical livestock-associated clonal complexes of *S. aureus*. ST9-MSSA colonizes humans and has been transmitted between humans and pigs in the United Kingdom (Armand-Lefevre et al., 2005), but further investigation of host tropism between humans and pigs is necessary to determine the origin of ST9 as an emerging livestock-associated *S. aureus* lineage.

The ST9 isolates investigated here are deficient in the type IV RM system, which may facilitate the transfer of foreign DNA. Deficiency in the type IV RM system has recently been observed in *S. aureus* prone to the acquisition of the *vanA* gene from enterococci (Corvaglia et al., 2010). Additionally, two novel transposon-like elements were found in the investigated isolates. The respective genes show a high degree of similarity to genes from coagulase negative staphyloccci or enterococci, and have not previously been reported for *S. aureus*. This would support the hypothesis that a nonfunctional RM system could enhance the uptake of foreign DNA by isolates in the ST9 lineage. And this hypothesis should

be confirmed experimentally further.

According to the nomenclature criteria for SCC*mec* types (IWG-SCC, 2009), the SCC*mec* elements in the two sequenced MRSA isolates belong to the type XII with a class C2 *mec* gene complex and *ccrC* gene complex. A similar structure for SCC*mec* was recently reported from a bovine ST9 MRSA in China and is consistent with dissemination of this SCC*mec* type in Chinese livestock (Wu et al., 2015). The genes encoded are similar to genes from coagulase-negative *Staphylococci*, a potential origin for this SCC*mec* element through their shared ecology (Meric et al., 2015). A relatively high frequency of *ccrC* positive SCC*mec* was found in *S. hominis,* which suggests that *S. hominis* could serve as a reservoir for this type of SCC*mec* for *S. aureus* (Mendoza-Olazaran et al., 2013). A pseudo (Ψ) SCC element with a truncated *ccrA* gene located in the left extremity of the SCC*mec* identified in this study showed high similarity to a ΨSCC element found in type IX SCC*mec* of a ST9 MRSA isolate from Thailand (Anukool et al., 2011; Lulitanond et al., 2013; Sinlapasorn et al., 2015).

The SCC*mec* structure identified in the present study implies multiple recombination events in this region of the *S. aureus* genome. As for other SCC*mec* types (Chlebowicz et al., 2013), the intact ΨSCC element was also found in the presently sequenced MSSA isolate. Combined with our phylogenetic analyses, this suggests that the SCC*mec* remnant in the MSSA strain may have been derived from a MRSA ancestor that lost the mobile region containing the *mec* and *ccr* gene complexes. Partial loss of SCC*mec* has been observed during a human infection (Chlebowicz et

al., 2010) and reported under specific circumstances, including absence of selective pressure imposed by antibiotics. Its excision from the chromosome would then result in a MSSA strain that could still carry a larger number of resistance determinants than usually found in native MSSA isolates (Donnio et al., 2005). The MSSA isolate investigated in the present study was multiply resistant and carried the same resistance genes as both investigated MRSA isolates except the *mecA* gene for methicillin resistance. The SCC*mec* remnant in the MSSA strain may also generate a false-positive result by real-time PCR assay for rapid dectection of MRSA directily from specimens (Arbefeville et al, 2011; Blanc et al, 2011). The new target strategies and interpretation might overcome the disadvantages of current assays (Becker et al, 2016).

In summary, the ST9 lineage of *S. aureus* seems to be phylogenetically distinct from other livestock-associated lineages, such as ST398. Our three livestock-associated Chinese ST9 isolates are deficient in the type IV RM system, which may permit increased acquisition of virulence factors, including SCC*mec,* pathogenicity islands and transposons originating from other species. Access to extended gene pools in livestock-associated isolates may serve as a reservoir of virulence and antibiotic resistance traits and pose a major public health risk of zoonotic infection in humans.

Acknowledgements

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Table 1. Characteristics of three *lsa(E)* positive *S. aureus* isolates.

Isolate name	Source	Sample type	MLST type(CCs)	*spa* type	*mecA*	*pvl*	MIC of QDA(mg/L)	*lsa(E)*	Antibiotic resistance phenotype*(24)
A69	pig	nose swab	9(CC9)	t899	+	-	8	+	FOX-CHL-TET-GEN-CIP-ERY-CLI -CTT-TRS-QDA
A71	pig	nose swab	9(CC9)	t899	+	-	8	+	FOX-CHL-TET-GEN-CIP-ERY-CLI -CTT-TRS-QDA
A187	pig	nose swab	9(CC9)	t899	-	-	8	+	CHL-TET-GEN-CIP-ERY-CLI -CTT-TRS-QDA

*FOX, cefoxitin; CIP, ciprofloxacin; ERY, erythromycin; TET, tetracycline; CLI, clindamycin; GEN, gentamicin; CTT, chlortetracycline; CHL, chloramphenicol; TRS, trimethoprim/sulfamethoxazole; QDA, quinupristin/dalfopristin;

Table 2. General properties of the genomes of the *S. aureus* ST9 isolates A69, A71 and A187 and *S. aureus* N315.

Element and characteristic	A69	A71	A187	N315[45]
Chromosome				
Size (bp)	2,844,421	2,849,873	2,786,770	2,813,641
G+C content (%)	32.80%	32.80%	32.80%	32.80%
No. of coding sequences	2779	2781	2671	2595
tRNA genes	55	59	54	62
Transposons				
Tn552	1	1	1	0
Tn554	0	0	0	5
Tn558	1	1	1	0
Others	2	2	2	0
Bacteriophages	1	1	0	1
SCC*mec*	1	1	0	1
Pathogenicity islands	2	2	1	3
Genomic islands	2	2	2	0
Plasmids				
Size (bp)	3,027	2,581	2,990	24,653
G+C content (%)	28.6	28.2	29.03	28.7

No. of coding sequences	2	2	2	29

Table 3. Summary of the major mobile genetic elements (MGEs) present in the three sequenced *S. aureus* ST9 isolates associated with virulence and antibiotic resistance.

	A69	A71	A187
Bacteriophage			
ØSa2	NI	NI	-
Genomic islands*			
γSaα	*set(11),lpl(7)*	*set(11),lpl(7)*	*set(11),lpl(6)*
γSaß	entertoxin(7), *lpl*(3)	entertoxin(8), *lpl*(3)	entertoxin(8), *lpl*(3)
Pathogenicity islands			
SaPIbov4-like	*vwb, scn,aadE*	*vwb, scn,aadE*	*vwb, scn,aadE*
SaPI6Δa	NI	NI	-
SCC*mec*	*mecA*	*mecA*	
Plasmid	NI	NI	NI
Transposons			
Tn552	*blaZ*	*blaZ*	*blaZ*
Tn552-like transposon	*blaZ*	*blaZ*	*blaZ*
Tn558	*fexA*	*fexA*	*fexA*
Transposon-like MGE with IS257	*lsa(E)lsa(E),lnu(B),aadE,tetL*	*lsa(E)lsa(E),lnu(B),aadE,tetL*	*lsa(E)lsa(E),lnu(B),aadE,tetL*

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transposase

NI, The MGE is present, but no virulence and antibiotic resistance gene was found.

*, the number of the virulence genes is indicated in parentheses.

Table 4. Transposon-like elements in the three sequenced *S. aureus* ST9 isolates and association with respective genes from other species

transposon-like element	ORFs	Protein	NCBI accession number*	Species(or Genus)
1	1	ArsR family transcriptional regulator	WP_002486904.1	*Staphylococcus epidermidis*
	2	permease	WP_020008110.1	*Salinicoccus albus*
	3	methyltransferase	WP_009384754.1	*Staphylococcus massiliensis*
	4	Tn552 transposase	WP_026066869.1	*Staphylococcus intermedius*
	5	ATP-binding protein	WP_019168760.1	*Staphylococcus intermedius*
	6	hypothetical protein	WP_019168761.1	*Staphylococcus intermedius*
2	1	TnpA	AAX38177.1	*Enterococcus casseliflavus*
	2	NimC/NimA family	WP_004193041.1	*Klebsiella pneumoniae, Escherichia coli, Salmonella, Enterobacteriaceae*
	3	partial DNA polymerase	WP_002303392.1	*Enterococcus faecium*
	4	partial,methyltransferase	WP_029751903.1	*Streptococcus suis*

*, the representative NCBI accession numbers are listed in the table.

Figure Captions

Fig. 1. Structure of the genetic environment of the *lsa*(E) gene of *S. aureus* isolates A69/A71/A187 and comparison with homologous structures. The genetic environment of the *lsa(E)* genes of the *S. aureus* isolates A69, A71 and A187 were compared to homologous regions of the *S. aureus* plasmid V7037(XJ560992), the *S. aureus* transposon *IS*257 (JQ861959), the *E. faecalis* plasmid pEF418(AF408195) and the *E. faecium* plasmid pXD4(KF421157). The arrows represent the positions and orientations of the genes. Similar regions in the different structures are indicated by grey shading.

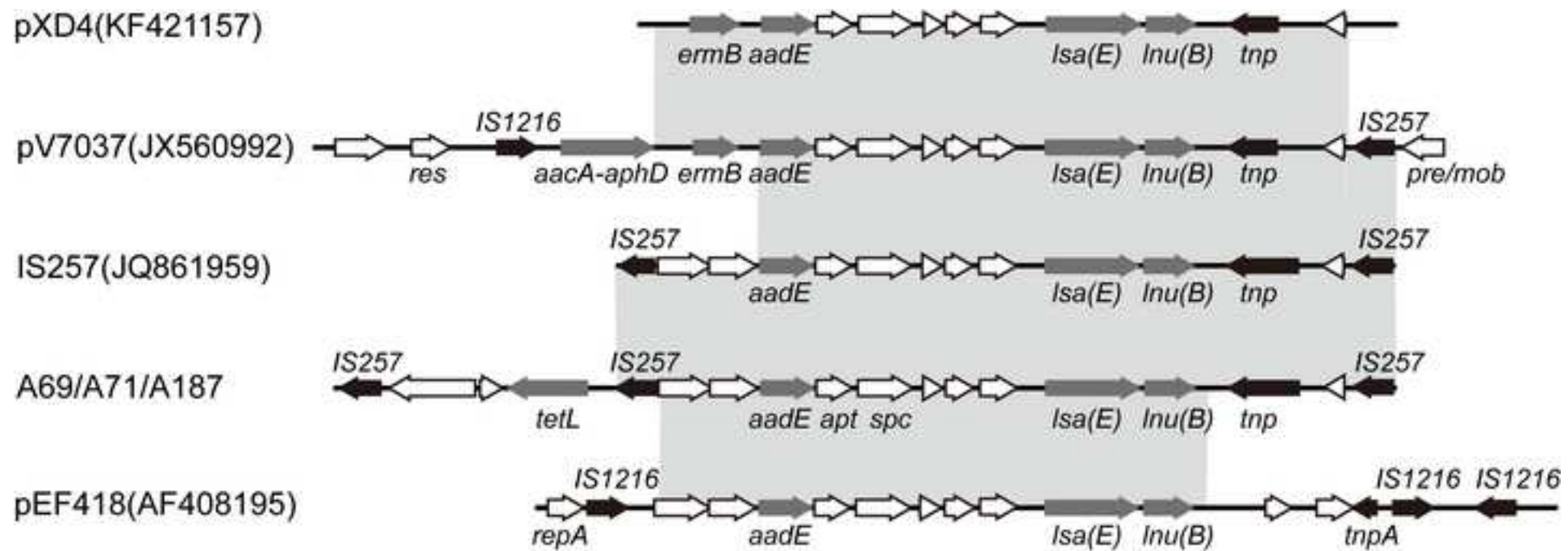**Fig. 2. Structure of the type XII SCC*mec* and pseudo (Ψ) SCC elements of the sequenced MRSA isolates (A69/A71) and homologous regions in the MSSA isolate A187 and *S. aureus* JCSC6690 (O2) from Thailand**. The SCC*mec* structures are illustrated based on the nucleotide sequences deposited in the GenBank database (AB705452 and AB705453). The red and blue arrowheads indicate the location of DR's with the respective sequences shown underneath. ORFs are indicated by arrows colored as follows: orange, insertion sequences; green, genes presumable acquired from other species; blue, *mecA*; pink, new allotype *ccrC*. The similarities in the different structures are indicated by grey shading.

Fig. 3. **Phylogenetic relatedness of the ST9 clone with other major clonal lineages of *S. aureus***. A phylogenetic tree was constructed from 2,596 genes (2,814,816 bp) shared between all 184 isolates available on NCBI using an approximation of the maximum-likelihood algorithm, implemented in FastTree2. Leaves on the tree are colored by the isolate's original source of isolation: 3 isolates from pigs (this study); 174 isolates from human origin; isolates from poultry origin; 3 isolates from bovine origin; 3 isolates

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from ovine origin; and 1 isolates from poultry origin. ST9 pig isolates are genetically close

to human isolates.

Figure

pXD4(KF421157)

ermB aadE   lsa(E) lnu(B) tnp

pV7037(JX560992)

IS1216   IS257

res   aacA-aphD ermB aadE   lsa(E) lnu(B) tnp   pre/mob

IS257(JQ861959)

IS257   IS257

aadE   lsa(E) lnu(B) tnp

A69/A71/A187

IS257   IS257   IS257

tetL   aadE apt spc   lsa(E) lnu(B) tnp

pEF418(AF408195)

IS1216   IS1216 IS1216

repA   aadE   lsa(E) lnu(B)   tnpA

Figure