Characterization of Insertion Sequence IS\textit{S}au\textit{2} in the Human and Livestock-Associated \textit{Staphylococcus aureus}

Liangliang Wang\textsuperscript{1}, Huping Xue\textsuperscript{1}, Longping Li\textsuperscript{1}, Xin Zhao\textsuperscript{1,2*}

\textsuperscript{1} College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi Province, People’s Republic of China, \textsuperscript{2} Department of Animal Science, McGill University, Quebec, Canada

* xin.zhao@mcgill.ca

Abstract

Mobile genetic elements play important roles in evolution and diversification of bacterial genomes. IS\textit{S}au\textit{2} is 1660bp in length with terminal 5’-TG and CA-3’ dinucleotides and has two overlapping reading frames \textit{orfA} and \textit{orfB}. It has been found in a wide range of \textit{S}. \textit{aureus}, such as HA-MRSA252, LGA251, MRSA S0385 and ED133. To determine distribution of IS\textit{S}au\textit{2}, 164 \textit{S}. \textit{aureus} isolates from milk samples of mastitic cows from our laboratory and all the \textit{S}. \textit{aureus} strains from the National Center for Biotechnology Information (NCBI) database were screened for the presence of IS\textit{S}au\textit{2}. Next, in order to explore a potential relationship among \textit{S}. \textit{aureus} IS\textit{S}au\textit{2}-containing strains and isolates, a relationship among 10 IS\textit{S}au\textit{2}-positive \textit{S}. \textit{aureus} isolates and 27 IS\textit{S}au\textit{2}-positive \textit{S}. \textit{aureus} strains was investigated by a phylogenetic analysis. These IS\textit{S}au\textit{2} isolates and strains could be classified into four groups (A, B, C and D). The strains or isolates in Group D were all isolated from mammary glands, suggesting tissue specificity. All strains in Group B had an identical IS\textit{S}au\textit{2} derivative, termed IS\textit{S}au\textit{2}\textsubscript{1628}, with 32bp deletion at the 3’ terminus. IS\textit{S}au\textit{2}\textsubscript{1628} in strain ST398 from Group B was closely related to IS\textit{S}au\textit{2} in strain LGA251 from Group D.

Introduction

Insertion sequences (ISs) are the smallest autonomously mobile genetic elements, generally 700–2500 base pairs (bp) in size, and are widely distributed in both eukaryotic and bacterial genomes. ISs play an important role in genome evolution by gene inactivation, genome rearrangement, and genome reduction as well as activation or inactivation of neighboring gene expression [1]. They are abundant and affect the genomic plasticity and pathogenic potential of \textit{S}. \textit{aureus} [2]. \textit{S}. \textit{aureus} is both a commensal organism and a pathogen in humans. The anterior nares are the main ecological niche for \textit{S}. \textit{aureus} [3]. However, numerous other sites may be colonized, including the axillae, groin, and gastrointestinal tract [3]. Colonization provides a reservoir from which bacteria can be introduced when host defenses are breached, whether by shaving, aspiration, insertion of an indwelling catheter, or surgery, causing a number of
invasive [3]. *S. aureus* also colonizes and affects a range of other animals including cows, pigs and goats [4].

Among over twenty IS families [1], the IS3 family is a large group found in both Gram-negative and Gram-positive bacterial species [5]. The IS3 family can be further divided into six subgroups (IS150, IS407, IS51, IS3, IS2 and IS911). Most IS3 family members share a common sequence organization in gene products and structural features, including the terminal dinucleotide 5’-TG———CA-3’, a small upstream open reading frame (*orfA*) and a longer downstream open reading frame (*orfB*). The OrfAB transposase is a fusion protein produced from both open reading frames by programmed -1 translational frameshifting [6], with the sequence motif A_{6}G as the frameshifting region [7,8]. OrfA, the deduced gene product from different IS3-like elements, typically contains a helix-turn-helix (HTH) motif that is crucial to direct the specific binding of the transposase to terminal inverted repeats [9]. Within OrfB, there is a conserved amino acid motif DDE which shares a strong homology with the catalytic site of retroviral integrases [10,11]. ISSau2 has similar gene products and structural features like most IS3 family members and is classified into the IS150 group in the ISfinder database.

Previously we found ISSau2 in the A region of *S. aureus* isolate E48 sdrC gene, which was isolated from a bovine mastitic cow in Canada [12]. It was also found in the literature in a wide range of *S. aureus*, such as HA-MRSA252 [13], LGA251 [14], MRSA S0385 [15] and ED133 [16]. The copy number and insertion sites of ISSau2 have been studied by whole genome sequencing of these strains. There were five copies in MRSA252, three in LGA251, one in MRSA S0385 and seven in ED133. Bioinformatics analyses of ISSau2 insertion sites in various *S. aureus* genomes did not reveal nucleotide sequence specificity for target sites [17]. Additionally, the copy number of ISSau2 was different in the three major clades (the clonal types were typically ST30spa43 for clade 1, ST30spa19 for clade 2, and ST36spa16 or ST30spa33 for clade 3) of *S. aureus* clonal complex (CC) 30 [17]. Whether other *S. aureus* isolates and strains also contain ISSau2 is worthy to be investigated. In addition, the relationship among the isolates and strains containing ISSau2 deserves exploration.

The aim of this study was to determine distribution of ISSau2 and to explore a potential relationship among *S. aureus* strains and isolates containing ISSau2.

**Material and Methods**

**PCR screening of bovine mastitis-associated *S. aureus* isolates for ISSau2 element**

Genomic DNA was purified from 164 *S. aureus* isolates from milk samples of mastitic cows from our laboratory [18], using a Genomic DNA purification kit (Tiandz, Beijing, China). The presence of ISSau2 was determined using specific primer pairs (IS-F and IS-R, Table 1) and the size of the PCR product was 1660bp. The PCR conditions were as follows: denaturation at 95°C for 5 min, annealing at 58°C for 1 min, extension at 72°C for 2 min and final extension at 72°C for 7 min. The copy number of ISSau2 in each isolate was determined by real-time qPCR.

**Table 1. List of the primers and probes used in this study.**

| Primers | Sequences |
|---------|-----------|
| IS-F    | TGAATAGCCTCCCTTCGAAAGTCAGACA |
| IS-R    | TGAACGTCAACCCAGTCTAGTAGACCAATT |
| SP1     | ATTCGATTGGCGAGAGCTTGGGTGTTG |
| SP2     | GTAAGTAGTGCTTCATACCATGCGG |
| SP3     | TCGATGAAATTTGACACGACACGC |

doi:10.1371/journal.pone.0127183.t001
Sequence analysis

ISSau2 DNA segments from ISSau2-positive S. aureus isolates were cloned into pEASY-T5 and sequenced by Genscript (Nanjing, China). The BLAST software from NCBI (blastn) was used to determine the presence of ISSau2 in genomes of all published S. aureus using ISSau2 from the E48 isolate as the query sequence and Reference genomic sequences (refseq_genomic) as the database with default algorithm parameters (the database was accessed on May 12, 2014). For bioinformatics analysis, ISSau2 sequences from different S. aureus strains or isolates were aligned using the ClustalX program [19] and phylogenetic trees were then constructed employing the MEGA 5.2 program on a neighbor-joining algorithm and a maximum likelihood method [20]. The two methods produced similar results.

Genomic walking

Genomic DNA was purified from nine ISSau2-positive S. aureus isolates and E48 using a Genomic DNA purification kit (Genomic Walking Kit, Takara Biotechnology, Dalian, China). Three specific primers (SP1, SP2 and SP3 in Table 1) were designed from the known sequence of ISSau2 using the Primer 5 program. A three step PCR assay was performed according to manufacturer’s instructions.

Results

Distribution and copy numbers of ISSau2 in S. aureus

To determine the presence of ISSau2 in S. aureus, a PCR to amplify the transposase region (1565bp) was carried out using the genomes of 164 bovine mastitis-associated S. aureus isolates as templates. According to the PCR results, 5.49% of the S. aureus isolates (9 out of 164) were confirmed to contain ISSau2 (data not shown). A nucleotide BLAST (BLASTn) of ISSau2 was also performed, utilizing the Reference genomic sequences (http://www.ncbi.nlm.nih.gov/refseq/) database as templates. Based on the blast results (E-value is 0.0), 27 S. aureus strains were found to contain the ISSau2 insertion sequences. The copy numbers of ISSau2 in S. aureus isolates were determined by Real-time qPCR. There are less than three copies in all the S. aureus isolates containing ISSau2 (Table 2). On the basis of the whole genome sequences, the copy numbers of ISSau2 in 27 published ISSau2-positive S. aureus strains were also determined, ranging from 1 to 7 (Table 2).

Sequence diversity of ISSau2

In order to determine the diversity of ISSau2, ISSau2 PCR products from S. aureus isolates were sequenced and 13 SNPs were found. Coupled with the BLAST results of ISSau2, a phylogenetic tree was constructed (Fig 1). They were classified into four groups (Groups A, B, C and D) with 14 different ISSau2 sequence variants. Each sequence variant was defined as a sequence type. The strains or isolates in an ISSau2 sequence type shared identical ISSau2 sequences. As shown in Table 2, S. aureus strains in three groups (Groups A, B and C) all belonged to a same CC- CC398, CC30 and CC22, respectively while Group D consisted of CC133 strains and other singletons. Three strains in Group A were all ST22 isolated from humans. After aligning the amino acid sequences of ISSau2 in Group A strains with that in isolate E48 containing a functional transposase, a His to Arg amino acid replacement was identified in the HTH domain, which is responsible for binding of the transposase to DNA. Group B included 11 strains that had an identical ISSau2 sequence, ISSau2 1628, with 32 nucleotides deleted at the 3’ terminus. This mutated sequence missing the IRR could not be recognized by the ISSau2 transposase and is therefore presumably inactive in transposition. The strains in this group were isolated...
Table 2. ISSau2-positive *S. aureus* strains and isolates evaluated in this study.

| Strain      | Location       | Year  | MLST | CC  | Number of ISSau2 | Number of ISSau2 sequence type | SCCmec | Host & Disease                              | Accession     |
|-------------|----------------|-------|------|-----|------------------|--------------------------------|--------|---------------------------------------------|---------------|
| MRSA252     | Oxford, UK     | 1997  | ST36 | CC30 | 5                | 3                              | HA-MRSA | Human with Septicemia                        | NC_002952.2   |
| MN8         | Korea          | 1980  | ST30 | CC30 | 3                | 1                              | MSSA    | Human                                       | CM000952.1    |
| M1256       | ND             | ST30  | CC30 | 2    | 1                | ND                             | MSSA    | Human                                       | NZ_KB822114.1 |
| M0513       | ND             | ST36  | CC30 | 5    | 1                | MRSAII                         | Human   | NZ_KB821413.1                              |
| EMRSA-16    | UK             | 1998  | ST36 | CC30 | 1                | 1                              | MRSAII  | Human with Chest infections, Septicemia, Endocarditis | NZ_GG770521.1 |
| LCT-SA112   | China          | ND    | ST243| CC30 | 1                | 1                              | ND      | Human                                       | NZ_JH691956.1 |
| TCH60       | TX, USA        | -     | ST30 | CC30 | 2                | 2                              | CA-MRSA | Human                                       | NC_017342.1   |
| M0408       | ND             | ST431 | CC30 | 5    | 1                | MRSA                           | Human   | NZ_KB821331.1                              |
| M809        | Australia      | 1961  | ST30 | CC30 | 1                | 1                              | MRSA    | Human                                       | NZ_GG749305.1 |
| C101        | UK             | 1997  | ST30 | CC30 | 1                | 1                              | MSSA    | Human                                       | NZ_GG730124.1 |
| 55/2053     | UK             | 1955  | -    | -    | -                | -                              | -       | -                                           | -             |
| HO_5096_0412| Suffolk, UK    | 2005  | ST22 | CC22 | 1                | 1                              | EMRSA-15 | Human with Lethal neonatal infections       | NC_017763.1   |
| M1228       | ND             | ST22  | CC22 | 1    | 1                | MRSA                           | Human   | NZ_KB822377.1                              |
| 21310       | ND             | ST22  | CC22 | 1    | 1                | MRSA                           | Human   | NZ_AFNP00000000.1                          |
| S0385       | Netherlands    | 2006  | ST398| CC398| 1                | 1                              | CA-MRSA | Human with Endocarditis; Also infect swine and calves | NC_017333.1   |
| DR10        | Dominican      | 2007  | ST398| CC398| 1                | 1                              | MSSA    | Human with Abscess                          | NZ_AIDT0000000.1|
| 08BA02176   | Canada         | 2008  | ST398| CC398| 1                | 1                              | LA-MRSA | Human with Postoperative infection          | NC_018608.1   |
| S1          | France         | 2008  | ST398| CC398| 1                | 1                              | LA-MRSA | Bovine                                     | NZ_AUPS0000000.1|
| S94         | France         | 2009  | ST398| CC398| 1                | 1                              | CA-MRSSA| Human with Bloodstream infections          | NZ_AUPW0000000.1|
| S123        | Dutch          | 2010  | ST398| CC398| 1                | 1                              | LA-MSSA | Swine                                      | NZ_AUPU0000000.1|
| S130        | Netherlands    | 2010  | ST398| CC398| 1                | 1                              | LA-MRSA | Swine                                      | NZ_AUPT0000000.1|
| S100        | France         | 2010  | ST398| CC398| 1                | 1                              | HA-MSSA | Human with Bloodstream infections          | NZ_AUPV0000000.1|
| 21331       | ND             | ST398 | CC398| 1    | 1                | MSSA                           | Human   | with Septicemia, Pneumonia                 | NZ_AGTV01000007.1|
| 112808A     | Switzerland    | 2008  | ST398| CC398| 1                | 1                              | MRSA    | Bovine Mastitis                             | NZ_AHZK0000000.1|
| 71193       | NY, USA        | 2004  | ST398| CC398| 1                | 1                              | MSSA    | Human                                       | NC_017673.1   |
| LGA251      | UK             | 2007  | ST425| -    | 3                | 1                              | MSSA    | Bovine Mastitis                             | NC_017349.1   |
| ED133       | France         | 1997  | ST133| CC133| 7                | 2                              | MSSA    | Ovine Mastitis                              | NC_017337.1   |
| E48         | Canada         | 2011  | ND   | -    | 1                | -                              | MSSA    | Bovine Mastitis                             | -             |
| 5–14        | China          | 2012  | ST2683| -   | 2                | -                              | MSSA    | Bovine Mastitis                             | -             |
| 5–16        | China          | 2012  | ST2683| -   | 1                | -                              | MSSA    | Bovine Mastitis                             | -             |
| 8–5         | China          | 2012  | ST2683| -   | 2                | -                              | MSSA    | Bovine Mastitis                             | -             |
| 8–6         | China          | 2012  | ST2683| -   | 1                | -                              | MSSA    | Bovine Mastitis                             | -             |
| 8–9         | China          | 2012  | ST2683| -   | 2                | -                              | MSSA    | Bovine Mastitis                             | -             |

(Continued)
from humans or livestock. Group C had 11 strains that display seven ISSau2 sequence types, with 3 ISSau2 sequence types for MRSA252 and two ISSau2 sequence types for TCH60. There were 0–4 SNPs in ISSau2 sequences in this group, when M0408 was used as a reference. These strains were all isolated from human infectious diseases. The 12 isolates or strains in Group D were tissue-specific, since they were all isolated from mammary glands with mastitis.

Table 2. (Continued)

| Strain | Location | Year | MLST | CC | Number of ISSau2 sequence type | SCCmec | Host & Disease | Accession |
|--------|----------|------|------|----|--------------------------------|--------|---------------|-----------|
| 8-19   | China    | 2012 | ST2683 | -  | 1                              | MSSA   | Bovine Mastitis | -         |
| 9-17   | China    | 2012 | ST2683 | -  | 2                              | MSSA   | Bovine Mastitis | -         |
| 9-18   | China    | 2012 | ST2683 | -  | 1                              | MSSA   | Bovine Mastitis | -         |
| 9-19   | China    | 2012 | ST2683 | -  | 2                              | MSSA   | Bovine Mastitis | -         |

“Number of ISSau2”: the copy number of ISSau2 in the according strain or isolate.

“Number of ISSau2 sequence type”: the number of ISSau2 sequence type in one S. aureus strain or isolate.

“MRSA”: Methicillin-resistant Staphylococcus aureus.

“HA-MRSA”: Healthcare-Associated Methicillin Resistant Staphylococcus aureus.

“CA-MRSA”: Community-associated methicillin-resistant Staphylococcus aureus.

“LA-MRSA”: Livestock-associated methicillin-resistant Staphylococcus aureus.

“MSSA”: Methicillin Susceptible Staphylococcus aureus.

doi:10.1371/journal.pone.0127183.t002

![Fig 1. Relationship among the ISSau2 containing S. aureus strains and isolates.](image)

The phylogenetic tree of ISSau2 gene was constructed by the MEGA software (version 5.2) using default parameters. Numbers at nodes are levels of bootstrap support (percentages) based on a neighbor-joining analysis of 1,000 resampled datasets using the Maximum Composite Likelihood method. Four groups (A, B, C and D) of 27 S. aureus strains and 10 isolates were indicated. The strains or isolates in a same branch shared identical ISSau2 sequences.
Insertion positions of ISSau2 in ISSau2-S. aureus

To define accurate insertion sites of ISSau2 in the genomes of S. aureus isolates, 5’ genomic walking was conducted in the genomes of 8 S. aureus isolates from this study. The PCR products were sequenced. Sequence analysis indicated that ISSau2 targets included: a gene encoding a hypothetical phage protein in S. aureus ED98, a gene encoding a putative membrane protein in S. aureus LGA251 and a gene encoding a membrane-embedded lipoprotein precursor in S. aureus RF122 (data not shown).

Our analysis of ISSau2 and its adjacent sequences in ISSau2-positive S. aureus strains also indicated that ISSau21628 in strain ST398 from Group B was closely related to ISSau2 of LGA251 in Group D, as shown in Fig 2. In the genome of LGA251, a hypothetical protein containing a hydrolase domain (indicated as A) was located before a putative membrane protein (as B) and a putative short chain dehydrogenase (as C). An ISSau2 sequence was found in front of the A/B/C proteins, with a distance of about 2200bp (Fig 2). Similar A/B/C proteins were only found in the genome of strain ST398 and ISSau2 was located between protein A and protein B.

Discussion

This study assessed the presence of ISSau2 in 164 S. aureus isolates from milk samples of mastitic cows in our laboratory and all the S. aureus strains from the National Center for Biotechnology Information (NCBI) database. Twenty seven S. aureus strains and 10 isolates were found to contain ISSau2 and these isolates and strains could be classified into 4 groups with certain specificities. In particular, those in group D were all isolated from mammary glands therefore exhibiting a strong tissue-specificity. In addition, the isolates used in this study from Shaanxi province in China could be classified in the same group as a Canadian isolate E48 and a UK-sourced strain LGA251. To the best of our knowledge, none of other strains or isolates in the other 3 groups was isolated from mammary glands. Group C strains were all from human sources suggesting host-specificity. Additionally, while Group A strains were also isolated from human infections, group members shared an ISSau2 sequence with a mutation in the HTH domain, different from strains in Group C.

It is interesting that the strains in Group B contained a short version of ISSau2 (ISSau21628) with the 32bp sequence of its 3’ terminal deleted. In addition, the adjacent genes of ISSau2 in strain ST398 of Group B and strain LGA251 were identical (Fig 2). We hypothesize that the relationship between strain ST398 and LGA251 is close. Using the ISSau2 in LGA251 as a reference, the ISSau2 in strain ST398 is a mutation-rich region with 116 SNPs. S. aureus CC398 is both a livestock and human pathogen which poses a worldwide threat because of its ability to...
colonize and infect both humans and animals [21]. CC398 strains lack significant virulence genes and the lineage features a unique genetic background [21]. Because of the specificity of IS\textsubscript{Sau2}1628, it could act as a genetic marker for recognizing IS\textsubscript{Sau2}-positive CC398 lineage.

It is widely accepted that transposition must be maintained at a low level due to its detrimental effect on the stability of the host genome [22]. Insertion elements have mechanisms to attenuate their activation. Expression of OrfA/B protein, produced by programmed -1 translational frameshifting, can act as a negative regulator of IS\textsubscript{Sau2} transposition, by competing for DNA binding/catalysis site of OrfAB to indirectly reduce the transposase activity [23]. Moreover, the frequency of frameshifting is also maintained at a low level, approximately 50% in the case of IS\textsubscript{150} [24] and 15% for IS\textsubscript{911} [8]. In addition, the copy number of IS\textsubscript{Sau2} of different \textit{S. aureus} strains was low, ranging from 1 to 7 copies. In particular, the copy number of IS\textsubscript{Sau2} in our bovine mastitis-associated \textit{S. aureus} isolate was less than three (Table 2). These may contribute to maintaining a low level of transposition of IS\textsubscript{Sau2} in nature.

Acknowledgments

We would like to thank Xubo Zhi for the gift of vectors. We are grateful to Zhiqiang Zhang and Pilong Liu for technical assistance. This study was financially supported by a grant from National Science Foundation of China (31372282), the China Thousand Talents program, and a University Scientific Research Fund project (Fund No. Z111021305). This work was also partially supported from a discovery grant by Natural Science and Engineering Research Council of Canada.

Author Contributions

Conceived and designed the experiments: XZ HX LW. Performed the experiments: LW LL. Analyzed the data: HX LW XZ. Contributed reagents/materials/analysis tools: LL HX. Wrote the paper: XZ LW HX.

References

1. Siguier P, Gourbeyre E, Chandler M. (2014) Bacterial insertion sequences: their genomic impact and diversity. FEMS Microbiology Reviews: 0.1111/1574-6976.12067.
2. Kuroda M, Ohta T, Uchiyama I, Baba T, Yuzawa H, Kobayashi I, et al. (2001) Whole genome sequencing of meticillin-resistant \textit{Staphylococcus aureus}. Lancet 357: 1225–1240. PMID: 11418146
3. Gordon RJ, Lowy FD. (2008) Pathogenesis of meticillin-resistant \textit{Staphylococcus aureus}. Clinical infectious diseases 46: S350–S359. doi: 10.1086/533591 PMID: 18462090
4. Baptiste KE, Williams K, Williams NJ, Wattret A, Clegg PD, Dawson S, et al. (2005) Methicillin-resistant staphylococci in companion animals. Emerging Infectious Diseases 11: 1942–1944. PMID: 16485485
5. Mahillon J, Chandler M. (1998) Insertion sequences. Microbiology and Molecular Biology Reviews 62: 725–774. PMID: 9729608
6. Chandler M, Fayet O. (1993) Translational frameshifting in the control of transposition in bacteria. Molecular Microbiology 7: 497–503. PMID: 8384687
7. Sekine Y, Eisaki N, Ohtsubo E. (1994) Translational control in production of transposase and in transposition of insertion sequence IS3. Journal of Molecular Biology 235: 1406–1420. PMID: 8107082
8. Polard P, Prere MF, Chandler M, Fayet O. (1991) Programmed translational frameshifting and initiation at an AUU codon in gene expression of bacterial insertion sequence IS911. Journal of Molecular Biology 220: 465–477. PMID: 1660923
9. Rousseau P, Tardin C, Tolou N, Salome L, Chandler M. (2010) A model for the molecular organisation of the IS911 transpososome. Mobile DNA 1: 16. doi: 10.1186/1759-8753-1-16 PMID: 20553579
10. Asante-Appiah E, Skalka AM. (1997) Molecular mechanisms in retrovirus DNA integration. Antiviral Research 36: 139–156. PMID: 9477115
11. Izsák Z, Khare D, Behlke J, Heinemann U, Plasterk RH, Ivics Z. (2002) Involvement of a bifunctional, paired-like DNA-binding domain and a transpositional enhancer in Sleeping Beauty transposition. Journal of Biological Chemistry 277: 34581–34588. PMID: 12082109

12. Xue H, Lu H, Zhao X. (2011) Sequence diversities of serine-aspartate repeat genes among Staphylococcus aureus isolates from different hosts presumably by horizontal gene transfer. Plos One 6: e20332. doi: 10.1371/journal.pone.0020332 PMID: 21625460

13. Holden MT, Feil EJ, Lindsay JA, Peacock SJ, Day NP, Enright MC, et al. (2004) Complete genomes of two clinical Staphylococcus aureus strains: Evidence for the rapid evolution of virulence and drug resistance. Proceedings of the National Academy of Sciences of the United States of America 101: 9786–9791. PMID: 15213324

14. Garcia-Alvarez L, Holden MT, Lindsay H, Webb CR, Brown DF, Curran MD, et al. (2011) Meticillin-resistant Staphylococcus aureus with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infectious Diseases 11: 595–603. doi: 10.1016/S1473-3099(11)70126-8 PMID: 21546353

15. Schijffelen MJ, Boel CH, van Strijp JA, Fluit AC. (2010) Whole genome analysis of a livestock-associated meticillin-resistant Staphylococcus aureus ST398 isolate from a case of human endocarditis. BMC Genomics 11: 376. doi: 10.1186/1471-2164-11-376 PMID: 20546353

16. Guinane CM, Ben Zakour NL, Tormo-Mas MA, Weinert LA, Lowder BV, Cartwright RA, et al. (2010) Evolutionary genomics of Staphylococcus aureus reveals insights into the origin and molecular basis of ruminant host adaptation. Genome Biology and Evolution 2: 454–466. doi: 10.1093/gbe/evq031 PMID: 20624747

17. McGavin MJ, Arsic B, Nickerson NN. (2012) Evolutionary blueprint for host- and niche-adaptation in Staphylococcus aureus clonal complex CC398. Frontiers in Cellular and Infection Microbiology 2: 48. doi: 10.3389/fcimb.2012.00048 PMID: 22919639

18. Li LP, Zhou LX, Wang LH, Xue HP & Zhao X. (2015) Characterization of meticillin-resistant and -susceptible staphylococcal isolates from bovine milk in Northwestern China. PLoS ONE 10: e0116699. doi:10.1371/journal.pone.0116699 PMID: 25756992

19. Thompson JD, Gibson TJ, Plewniak F, Jeaenmougin F, Higgins DG. (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25: 4876–4882. PMID: 9396791

20. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28: 2731–2739. doi: 10.1093/molbev/msr121 PMID: 21546353

21. Jamroz D, Mcויder M, Butaye P, Coldham NG. (2012) Comparative genotypic and phenotypic characterisation of meticillin-resistant Staphylococcus aureus ST398 isolated from animals and humans. Plos One 7: e404458.

22. Doolittle WF, Kirkwood TB, Dempster MA. (1984) Selfish DNAs with self-restraint. Nature 307: 501–502.24. PMID: 6320009

23. Sekine Y, Izumi K, Mizuno T, Ohtsubo E. (1997) Inhibition of transpositional recombination by OrfA and OrfB proteins encoded by insertion sequence IS3. Genes to Cells 2: 547–557. PMID: 9413996

24. Vögele K, Schwartz E, Welz C, Schiltz E, Rak B. (1991) High-level ribosomal frameshifting directs the synthesis of IS150 gene products. Nucleic Acids Research 19: 4377–4385. PMID: 1653413