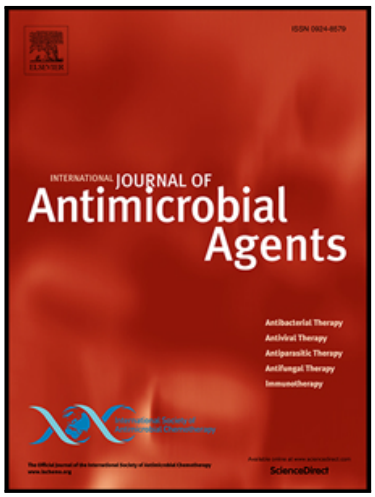

Genomic analysis of Staphylococcus aureus along a pork production chain and in the community, Shandong Province, China

Chengtao Sun , Baoli Chen , Anette Hulth , Stefan Schwarz , Xing Ji , Lennart E. Nilsson , Shizhen Ma , Qiang Sun , Zhenwang Bi , Yang Wang , Zhenqiang Bi , Congming Wu , Stefan Börjesson

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Highlights

- The first "One Health" molecular epidemiological survey of *S. aureus* along a pork production chain and in the surrounding community in Asian counties.
- The first study providing large-scale whole genome sequencing data of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) CC9.
- An early report of LA-MRSA ST398 and a novel LA-MRSA CC9 variant ST3597 from pigs in China.
- Dissemination of LA-MRSA CC9 was confirmed between various segments along the pork production chain.

Genomic analysis of *Staphylococcus aureus* along a pork production chain and in the community, Shandong Province, China

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Running title: *Staphylococcus aureus* along and around the pork production chain

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Abstract

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) is an increasingly important public health concern worldwide; however, data on LA-MRSA from Asian countries is scarce. Here, we performed a comprehensive molecular epidemiological survey of *S. aureus* along a pork production chain and in the community in Shandong Province, China. We used *spa*-typing and whole-genome sequencing to survey the occurrence and potential transmission of *S. aureus* in various sectors, including 899 pig samples (snout or skin swabs, carcass swabs and pork portions), 845 human nasal samples and 239 environmental samples from commercial farms, a slaughterhouse, a pork market and the surrounding community. MRSA was detected in higher frequencies in samples from two commercial pig farms (pigs, 49%; farm workers, 64%; environmental samples, 16%), than in samples from the slaughterhouse (fatteners, 8.2%; carcasses, 1.1%; operation workers, 0%; environmental samples, 3.8%), the pork market (pork, 14%; sellers, 0%) or individuals in the community (6.8%). There were significant differences in population structures, antimicrobial susceptibility profiles, and the presence of resistance and virulence genes between human- and pig-associated isolates. The phylogenetic analysis confirmed the dissemination of LA-MRSA between various segments along the pork production chain. However, MRSA of the same sequence type was not found to be disseminated between the commercial farms and the surrounding communities. Furthermore, we observed one MRSA ST398 and detected a novel CC9 variant ST3597 within the chain. The high MRSA carriage rates and the emergence of a new MRSA CC9 variant identified in this study highlight the necessity and importance of MRSA surveillance.

Keywords: LA-MRSA; China; Pork production chain; Community; WGS

1. Introduction

The global epidemic of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) among pigs has become a recognised public health concern [1-4]. In China, the occurrence and epidemiology of LA-MRSA have not been extensively studied compared to European countries. However, one major difference noted in China is that the LA-MRSA identified mainly belong to the clonal complex (CC) 9 [4], which is in contrast to Europe where LA-MRSA CC398 dominates [5]. CC9 isolates were first reported in pigs and people

with occupational contact to pigs in 2008 in China [6,7], but since then only a limited number of studies have described CC9 from livestock or slaughterhouses in China [4,8]. However, these studies do not generally provide in-depth molecular data, which are needed to understand the relatedness and adaption of LA-MRSA CC9 within the pig industry and other settings. Since more than half of the world's pig stock (~435 million) resides in China [9], these animals could represent the largest LA-MRSA reservoir in the world. It is therefore important to gain insight into the occurrence and potential spread of LA-MRSA in the pork production, its environment and surrounding community in China.

To improve the knowledge about the LA-MRSA situation in China, a cross-sectional study investigating both MRSA and methicillin-susceptible *S. aureus* (MSSA) was conducted. The study included samples from a typical Chinese pork production chain, operation workers, the environment, and surrounding community residents in Shandong Province, China. To give insight into the epidemiology of MRSA and MSSA from pig to pork, in the environment and surrounding community, we applied *spa*-typing followed by WGS and bioinformatic analysis.

2. Materials and Methods

2.1 Study population and sample collection

In April 2016, samples were collected from a typical commercial pork production chain attached to a local pork producing company in Shandong, the fourth-largest pig-producing province in China in 2016 [10]. The production chain was part of a vertically integrated system, meaning that pigs at commercial farms are produced exclusively for a local slaughterhouse and the pork wholesale market. Samples were collected from two pig farms, designated farm A and farm B (~4.5 km apart) with capacities of 1,500 and 3,000 pigs, respectively, a slaughterhouse, processing 1,000-1,500 pigs/day, and a pork wholesale market. Samples were also collected in three communities adjacent to the investigated pork production chain, community 1, ~0.5 km to the slaughterhouse; community 2, ~1.5 km to farm B; and community 3, ~2.0 km to farm A. Each community consisted of ~150 households and based on an estimated detection rate of 10% (from local Center for Disease Control and Prevention), 40 households were randomly contacted. In the end, ~20 households per community participated. From each household, a nasal swab was collected from one adult volunteer (Figure S1).

For pig snout and human nasal swabs, the ESwab^{TM} System (Copan, Brescia, Italy) was used according to the manufacturer's instructions. Snout swabs were randomly collected from sows (n = 137), weaners (n = 91) and growers (n = 70) from the farms with 1-3 pigs per pen and from fatteners (n = 85, pooled sample from three pigs) at the slaughterhouse. Carcass swabs (n = 98, pooled sample from three pigs) were collected after scalding and dehairing, but prior to further processing at the slaughterhouse. Pork portions (n = 14) of at least 50 g were collected from the pork wholesale market and transported in aseptic sampling bags (Hope Bio-Technology Co., Qingdao, China). Environmental samples comprised effluents (~40 mL; farms, n = 33; slaughterhouse, n = 8), air (90-150 cm off the ground; farms, n = 27; slaughterhouse, n = 8) and soil (~40 g, undisturbed soil, 5-10 cm of top layer out of each pig pen at farms, n = 40), as well as surface swabs of 20 cm^2 of the corridor floors (farms, n = 40; slaughterhouse, n = 5) and walls (farm, n = 50; slaughterhouse, n = 5). If present surface samples of equipment (farms, n = 23) were collected from three locations within the pig pen, drinking water taps, feeding troughs, and ventilation system, respectively. Air samples were collected in each pig pen in the farm and along the slaughtering line in the slaughterhouse using a Sennon JWL-S6 air sampler (Beijing, China) with CHROMagar MRSA and CHROMagar *Staphylococcus aureus* (CHROMagar Company, Paris, France) plates, respectively (Table S1, Figure S1).

Human and porcine samples from a previous study located in 12 rural villages neighbouring the production chain (slaughterhouse to the nearest and farmost village: ~17–28 km) were also included in this study [11], for comparison of the epidemiology of MRSA and MSSA from humans with and without backyard pig farms, as well as from pigs between the commercial farms and backyard farms. These samples were collected in July 2015 from pigs (skin swabs behind the ear, n = 404) and humans (nasal swabs, n = 753), from 245 and 753 households, respectively (Table 1, Figure S1) [12].

2.2 Cultivation and verification of MSSA and MRSA

From ESwab tubes, 0.2 mL of transport liquid was transferred to 1.3 mL of 7.5% sodium chloride broth (Land Bridge, Beijing, China) and incubated overnight at 35°C, after which cultures were plated on CHROMagar MRSA and CHROMagar *Staphylococcus aureus* (CHROMagar Co.), respectively. One single putative MRSA and *S. aureus* colony was selected from the respective plate and sub-cultured on Baird-Parker agar (Land Bridge) overnight at 35°C. The suspected colonies were confirmed as *S. aureus* using matrix-assisted

laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) (Bruker, Bremen, Germany), with the suspected MRSA confirmed by PCR [13].

Effluents, soil, pork portions and surface samples were pre-enriched in BHI broth (Land Bridge) for 2 h at 35°C to collect suspected *S. aureus* isolates within those fractal samples. A total of 0.2 mL of pre-enriched BHI broth was transferred to 1.3 mL of 7.5% sodium chloride broth for further enrichment and was subsequently processed as the samples collected with Eswab.

2.3 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using broth-microdilution and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) documents VET08 [14] and M100-S28 [15]. *S. aureus* ATCC 29213 was used as a control strain.

2.4 Genetic characterization and phylogenetic analysis

MRSA and MSSA isolates identified were subjected to *spa* typing [16], and *spa*-type was assigned using *spa*-plugin in BioNumerics v7.6 (Applied Maths, Sint-Martens-Latem, Belgium). Minimum spanning trees based on the *spa*-types were constructed in BioNumerics. Based on *spa*-types and background information regarding source and location, representative MSSA and MRSA isolates were selected for WGS and sequenced using Illumina technology [17]. Multi-locus sequence types (MLST) were assigned according to the *S. aureus* MLST database [18] by mapping reads to alleles using SRST2 [19]. Antimicrobial resistance (AMR) and virulence genes were screened using a mapping approach implemented in SRST2 against the ResFinder 2.1 (17-Feb-2017) [20] and VirulenceFinder 1.6 (18-Feb-2016) [21] databases. De-novo assembly was performed in CLC Genomics Workbench 9 (CLC Bio, Aarhus, Denmark) with de Bruijn graphs. Sequence data were submitted to GenBank and are registered under BioProject accession no. PRJNA433074. Based on the draft genome sequences, a core genome single nucleotide polymorphism (SNP)-based maximum likelihood (ML) phylogenetic tree was constructed for all sequenced MSSA and MRSA isolates, with the genome of ST9 LA-MRSA QDCD9 used as a reference. The tree was constructed using Parsnp in the Harvest package [22] with default parameter settings and visualised using iTOL [23].

2.5 Definitions and statistical analysis

MRSA isolates were classified as human or pig-associated based on the dominant origin of each ST/CC, while MSSA isolates were classified based solely on their origin. Transmission events were defined as isolates with core genome-SNP divergence of less than 20 [24-26].

Descriptive and comparative analyses were performed in GraphPad Prism 7.0 (GraphPad Software, CA, USA), statistical analyses were performed in SPSS 25.0 (IBM, Chicago, USA). Differences in the total number of AMR genes, virulence genes and the number of resistance traits among human- and pig-associated isolates were assessed using the Wilcoxon test. Fisher's exact test was used to test whether differences in frequencies of individual genes encoding resistance or virulence and drug resistance phenotypes were significant.

2.6 Ethical approval:

A signed consent form was obtained from all human participants, and animal sampling was conducted in accordance with the principles of the Beijing Municipality Review of Welfare and Ethics of Laboratory Animals (BAOLA 2005). Ethical approval was given by The First Affiliated Hospital of Zhejiang University (2015#185 and 2015#283).

3. Results

3.1 Prevalence of MRSA in the pork production chain and the community

In total, 223 MRSA and 199 MSSA isolates were recovered from 1,983 samples collected from various sources and locations (Table S1), with all MRSA carrying the *mecA* gene. MRSA isolates were detected in pigs from commercial farms (49%, 146/298), the slaughterhouse (fatteners, 8.2%, 7/85; carcass, 1.0%, 1/91) and pork meat (14%, 2/14) from the wholesale pork market (Figure 1a). In farm B, 80% (132/166) of pigs were positive for MRSA, while on farm A, 11% (14/132) of pigs were MRSA-positive (Table S1). Workers in the production chain also carried MRSA; farm workers (64%, 9/14; farm A, 33%, 2/6; farm B, 88%, 7/8), slaughterhouse workers (0%, 0/16) and pork market sellers (0%, 0/3), with an average nasal carriage of 27% (9/33). MRSA detection rate of residents from the surrounding community was 6.8% (4/59), and in rural villages, it was 1.7% (13/753) (Figure 1b, Table S1) [11].

Environmental samples, effluent, air and surface samples from the commercial farms, were also MRSA-positive with average detection rates of 5.0% (7/140) and 36% (26/73) at farm A and B, respectively. Most environmental samples from the slaughterhouse tested negative for both MRSA and MSSA, except for one MRSA-positive effluent sample (Figure 1c, Table S1).

3.2 Population structure of MRSA and MSSA

A total of 51 *spa*-types were identified among the 422 *S. aureus* isolates, with MSSA isolates (47 *spa*-types) showing a greater diversity than MRSA (4 *spa*-types) (Figure 2a, Table S1). Most MRSA isolates belonged to t899 (93%, 207/223), with the remaining MRSA isolates belonging to t437, t034 and t3527. MRSA *spa*-type t899, t437 and t034, but not t3527 were also detected among MSSA isolates (Figure 2a).

MRSA isolates of human origin were predominantly t899 (54%, 14/26) and t437 (39%, 10/26) (Figure 2b), while the clear majority of pig MRSA isolates were t899 (98%, 159/162) (Figure 2c). Of the MSSA isolates, the top four *spa*-types corresponded to 59% (117/199) of all MSSA isolates; t034 (35%, 70/199), t899 (13%, 25/199), t458 (6.0%, 12/199) and t002 (5.0%, 10/199). The 87 human MSSA isolates belonged to 41 different *spa*-types (Figure 2b), while the 99 porcine MSSA isolates belonged to 12 *spa*-types (Figure 2c).

A total of 128 isolates, 92 MRSA belonging to 4 *spa*-types and 36 MSSA belonging to 8 *spa*-types, were subjected to WGS (Figure S2a, Table S2,). Most MRSA isolates belonged to ST3597-t899 (62%, 57/92), followed by ST9-t899 (20%, 18/92) and ST59-t437/t034/t3527 (16%, 15/92), with one t437 isolate identified as ST398. Most MSSA isolates were ST398 (53%, 19/36), followed by ST9 (22%, 8/36), ST188 (14%, 5/36), ST59 (2.8% 1/36) and ST3597 (2.8% 1/36). Three isolates belonged to new STs, one MRSA isolate, a single locus variant of ST3597, and two MSSA isolates, single locus variants of ST9 and ST59, respectively, which were assigned as ST5051, ST5052 and ST5053, respectively.

MRSA ST3597-t899 isolates were frequently identified in samples collected from commercial farms, including isolates both from pigs (98%, 48/49) and workers (78%, 7/9), while ST9-t899 isolates were predominantly obtained from rural backyard pigs (86%, 6/7) and the slaughterhouse (57%, 4/7). In contrast, ST59-t437 isolates were primarily found in samples from humans in rural villages (47%, 7/15) and in the community surrounding the production chain (27%, 4/15). MSSA isolates from commercial farms, slaughterhouse, pork

market and the rural villages were predominantly of the same MLST type, ST398, but representing a distinct set of *spa*-types (Table S2).

Core genome-based ML phylogenetic analysis of the 92 MRSA and the 36 MSSA isolates identified four groups, corresponding to STs identified as CC9, ST188, ST59 and ST398 (Figure 3). SNP divergence among various isolates was calculated from a total of 31,564 core-genome SNPs.

All MRSA and MSSA isolates from the surrounding community were phylogenetically separated from those in the production chain. However, indication of MRSA transmission along the production chain was observed. For example, ST3597-t899 MRSA isolate 2AY27 recovered on farm A was closely related to DY67-1 recovered from the slaughterhouse (19 SNPs difference). Further, within the commercial farms, an overlap of LA-MRSA was observed between pigs, farm workers, the environment and between the two farms. For example, ST3507-t899 MRSA AB4 recovered from a worker on farm A was closely related to BY2 of porcine origin on farm A (1 SNP difference). This was also observed on farm B, as ST3597-t899 MRSA 2AB6-1 (farm worker) and BY78 (weaner) were closely related (1 SNP difference). Also, on farm B, ST3597-t899 MRSA isolates 2AY7-1, 2AY20, 2AY36, 2AY41, and 2AY47 recovered from sows were closely related to isolates recovered from weaners (BY53, BY56-1, BY80, BY69 and BY68) (0–4 SNPs difference), and ST3597-t899 MRSA isolates BY54 and BY74 recovered from weaners were closely related to isolates recovered from growers (2CY8 and 2CY4) (0–2 SNPs difference). Further, we observed that ST3597-t899 MRSA isolate 2BA2-1 recovered from an air sample on farm B was closely related to the porcine isolate BY48-1 (1 SNP difference) of the same farm. In addition, ST3597-t899 MRSA isolate BY7 recovered from weaner on farm A was identical to isolates recovered from sows (2AY7-1 and 2AY20) on farm B (0 SNP divergence) (Figure 3).

Within the backyard farms, MRSA from humans at two households in the rural villages (AK046, ST59-t437; AH022, ST9-t899) were closely related to those from pigs within the same household (YK046, ST59-t437; YH022, ST9-t899) (10–12 SNPs difference). Potential MSSA overlaps were also detected between individual humans within the same village, such as isolates AD057 (ST59-t437) and AD016 (ST59-t437) from village D (0 SNP difference), S1AH011 (ST9-t899) and S1AH053 (ST9-t899) from village H (0 SNP difference) (Figure 3).

3.3 Distribution of AMR and virulence genes in MRSA and MSSA

In total, 23 AMR genes and 24 virulence genes were detected among the isolates (Figure 4a and 4b). Acquired AMR genes were widespread in isolates of both human and pig origin. Except for the widely distributed resistance genes in this collection, such as *blaZ* and *norA*, the distribution of individual AMR genes varied among isolates of human- and pig-associated groups. Among the MRSA isolates, acquired AMR genes were more frequently detected in pig-associated isolates than human-associated isolates (median 12 vs 9; $p<1\times10^{-7}$) (Figure 4d). Aminoglycoside resistance genes, including *aacA-aphD*, *aadD* and *aadE*, were strongly associated with MRSA isolates from pigs (>95% of pig-associated isolates vs ≤40% of human-associated isolates; $p<1\times10^{-7}$) (Figure 4a). In contrast, *ant(6)-Ia* and *aph(3')-III* were more frequently detected in MRSA of human origin (73% of human-associated isolates vs. ≤ 2.6% of pig-associated isolates; $p<1\times10^{-9}$) (Figure 4a). Similarly, the tetracycline resistance gene *tet*(L) was strongly associated with MRSA of pig origin (99% of pig-associated isolates vs. 40% of human-associated isolates; $p<1\times10^{-7}$), while isolates of human origin were generally positive for *tet*(K) (60% of human-associated isolates vs. 7.8% of pig-associated isolates; $p<1\times10^{-4}$) (Figure 4a). The same was also observed for macrolide resistance genes. The gene *erm*(C) was associated with pig MRSA isolates (65% of pig-associated isolate vs. 40% of human-associated isolates; $p<0.01$), while *erm*(B) was found in 80% of human-associated isolates and in only 6.5% of pig-associated isolates ($p<1\times10^{-8}$) (Figure 4a). The genes *dfrG*, *fexA*, *lnu*(B) and *lsa*(E) coding for resistance to trimethoprim, phenicols, lincosamides and pleuromutilins-lincosamides-streptogramin A, respectively, were more prevalent in isolates of pig origin than in human isolates (>96% of pig-associated isolates vs. ≤40% of human-associated isolates; $p<1\times10^{-7}$). Of the MSSA isolates of pig origin, the median number of AMR genes was 10 per isolate, with six AMR genes detected per isolate from humans. Resistance genes *str*, *tet*(L), *tet*(M), and *lnu*(A) were detected at higher rates in MSSA isolates from pigs (mainly ST398 isolates, 11/16) than in human isolates ($p<0.05$), while no specific gene was associated with MSSA isolates of human origin (Figure 4a).

Differences of virulence genes in each isolate were also observed between isolates of pig- and human-associated group (MRSA, median 11.1 vs. 11.0, $p<1\times10^{-16}$; MSSA, median 8.3 vs. 9.2, $p<0.05$) (Figure 4e). Haemolysin-encoding genes *hlgA*, *hlgB* and *hlgC*, along with aureolysin-encoding gene *aur*, were present in all MRSA and MSSA isolates. However, staphylokinase gene *sak*, staphylococcal complement inhibitor *scn* and enterotoxin genes *seb*,

sek and *seq* were more prevalent in isolates from humans (>73% of human-associated isolates vs. <2.6% of pig-associated isolates; $p<1\times10^{-7}$), while the enterotoxin genes *seg*, *sei*, *sem*, *sen*, *seo* and *seu* were more common in isolates from pigs (100% of pig-associated isolates vs. <27% of human-associated isolates; $p<1\times10^{-7}$) (Figure 4b). Only *sak*, *scn* were associated with MSSA isolates from humans (37% of pig-associated isolates vs. <6.3% of human-associated isolates; $p<0.05$). Finally, one ST59-t189 MRSA isolate, recovered from a community resident, was *lukF-PV*- and *lukS-PV*-positive, and all five ST188-t189 MSSA isolates (four of human origin and one of pig origin) recovered from rural villages, were positive for *lukD* and *lukE*, with the *luk* genes coding for leucocidins. The isolates of pig origin were all negative for Panton Valentine leucocidin genes (Figure 4b).

3.4 Antimicrobial susceptibility profiles of MRSA and MSSA

Compared to MRSA isolates from humans, MRSA isolates from pigs were resistant to a larger number of antimicrobial agents (median 7.6 vs. 5.1; $p<1\times10^{-7}$) (Figure 4f). Resistance to florfenicol, gentamicin, tetracycline, tiamulin and ciprofloxacin were associated with the MRSA isolates from pigs (81% of pig-associated isolates vs. ≤60% of human-associated isolates; $p<0.01$) (Figure 4c). Erythromycin and clindamycin resistance were commonly observed in MRSA isolates regardless of their origin (human, 100%, 93%; pig, 97%, 97%) (Figure 4c). No specific phenotype of resistance could be linked to the origin of MSSA isolates. All isolates tested were susceptible to linezolid, vancomycin and fusidic acid (Figure 4c).

4. Discussion

To our knowledge, this is the first study in China to assess the epidemiology and transmission of *S. aureus* in a pork production chain, operation workers, the environment and surrounding community. Along the pork production chain, we detected MRSA-CC9 from the commercial farms, the slaughterhouse, as well as at the wholesale pork market, but the occurrence of MRSA decreased along the chain. The results indicate that MRSA-CC9 can spread along the pork production chain, but that the potential spread between the commercial farms and the surrounding community and villages is limited as no closely related MRSA-CC9 were identified in the community or villages. The MRSA identified in humans in the surrounding community and villages belonged instead to ST59-t437, which is a

common community-associated MRSA (CA-MRSA) strain in China [27]. The high MRSA carriage among farm workers (64%) combined with a lack of MRSA-CC9 isolates in the community is in accordance with an earlier Chinese study that showed general population without livestock contact are less likely to carry MRSA-CC9 [28]. The high occurrence in pig production is still worrisome because in Europe an increased incidence of LA-MRSA-CC398 has been observed among humans without livestock contact [1-3].

A large difference in the prevalence of MRSA among pigs of the two investigated farms, 80% (farm B) and 11% (farm A), was observed, potentially connected to the larger farm size of farm B [29]. This observation was not surprising as a previous study in Shandong Province described great variations of MRSA-positive pigs between different farms, 0–45% [8]. Other Chinese studies have also shown that the occurrence of MRSA differs geographically, with estimates ranging from 3.6% (Ningxia) to 47% (Shanghai) [6, 8, 30].

Most MRSA isolates retrieved from the production chain was ST3597-t899, a single locus variant of ST9. ST3597 were widely distributed in the two commercial farms among pigs, farm workers, environmental samples, and occurred sporadically at the slaughterhouse (Figure S2b). In contrast, the MRSA-positive backyard pigs in the same area all carried ST9-t899 [11]. Furthermore, all ST3597 isolates were closely related and were phylogenetically separated from ST9 isolates (Figure S2b). These results indicate a recent clonal expansion of the ST3597 in farm A and farm B, rather than multiple introductions into these farms. To our knowledge, this is the first description of this specific CC9 variant in Chinese pig farms, although there is a previous report of another ST9 single locus variant, ST1376, from one pig farm [7].

In contrast to the MRSA situation, ST398 was the most frequent porcine and human MSSA sequence type identified in the current study. In China, the ST398 is primarily associated with community- and hospital-acquired MSSA infections in humans [27,31]. Similar to a previous finding [32], 4 of 7 ST398-MSSA isolated from humans carried genes related to the immune evasion gene cluster (IEC), while none of the twelve isolates from pigs carried these genes (Table S2). This indicates that two different ST398 populations are circulating in the area, one in humans and another one in pigs. Furthermore, one ST398-MRSA isolate (DY77) was identified in fatteners from the slaughterhouse in the current study. Interestingly, this ST398-MRSA belonged to the *spa*-type t437, which in this and other studies was associated with ST59 [11, 33]. Although MRSA-ST398 is uncommon

in pigs in China, there is one recent report of MRSA-ST398 from a pig farm in Southern China, but the respective isolates belonged to the *spa*-types t034 and t571 [34].

The occurrence of resistance genes in MRSA and MSSA pig isolates was generally higher than that in the isolates from humans. The resistance phenotypes also differed between isolates of human- and pig-associated groups, e.g. resistance to florfenicol, gentamicin, tetracycline, tiamulin and ciprofloxacin, and there was a concordance between AMR genotypes and phenotypes for most of our isolates. The differences in antimicrobial resistance between human and pig isolates could be due to differences in antimicrobial use. Interestingly, the tetracycline resistance gene *tet*(L) was associated with pig-associated MRSA isolates (99% of isolates), while both human- and pig-associated MRSA were negative for *tet*(M), which is a well-known genetic marker for livestock-associated *S. aureus* CC398 [32, 35]. The occurrence of *tet*(L), *erm*(B), together with the lack of IEC genes (*sak* and *scn*) in MRSA-CC9 may function similarly as markers for LA-MRSA in China. In addition, CC9-MRSA was also characterized by its high occurrence of staphylococcal enterotoxins coding genes (*seg*, *sei*, *sem*, *sen*, *seo* and *seu*) which are due to the enterotoxin gene cluster *egc* [36]. However, additional studies in CC9 from both human and animal populations are necessary to validate the markers observed in our study.

In the current study, we provide a comprehensive molecular epidemiology portrait of MRSA and MSSA along a typical Chinese commercial pork production chain and the potential overlap with the surrounding population. The WGS-based approach confirmed dissemination of CC9 between pigs, farm workers and environment within the commercial farms, between pigs in commercial farm and slaughterhouse along the pork production chain, as well as between pigs and humans within households, while it did not reveal any indications that MRSA and MSSA from the production chain are circulating in the surrounding community. Instead we show that in this Chinese region there are today two separate MRSA populations, one connected to pigs with mainly MRSA CC9 carrying *tet*(L) but lacking *erm*(B) and ICE genes, and one in humans with primarily MRSA ST59 carrying *erm*(B) and IEC genes but lacking *tet*(L), also two different MSSA ST398 populations are circulating in humans and pigs differing in IEC genes. To better understand the occurrence of LA-MRSA in pigs in China and to monitor its epidemiological changes, surveillance, as well as further research studies in pigs, humans and their environment, are needed.

Declarations

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Competing Interests: None declared.

Ethical Approval: Ethical approval was given by The First Affiliated Hospital of Zhejiang University (2015#185 and 2015#283).

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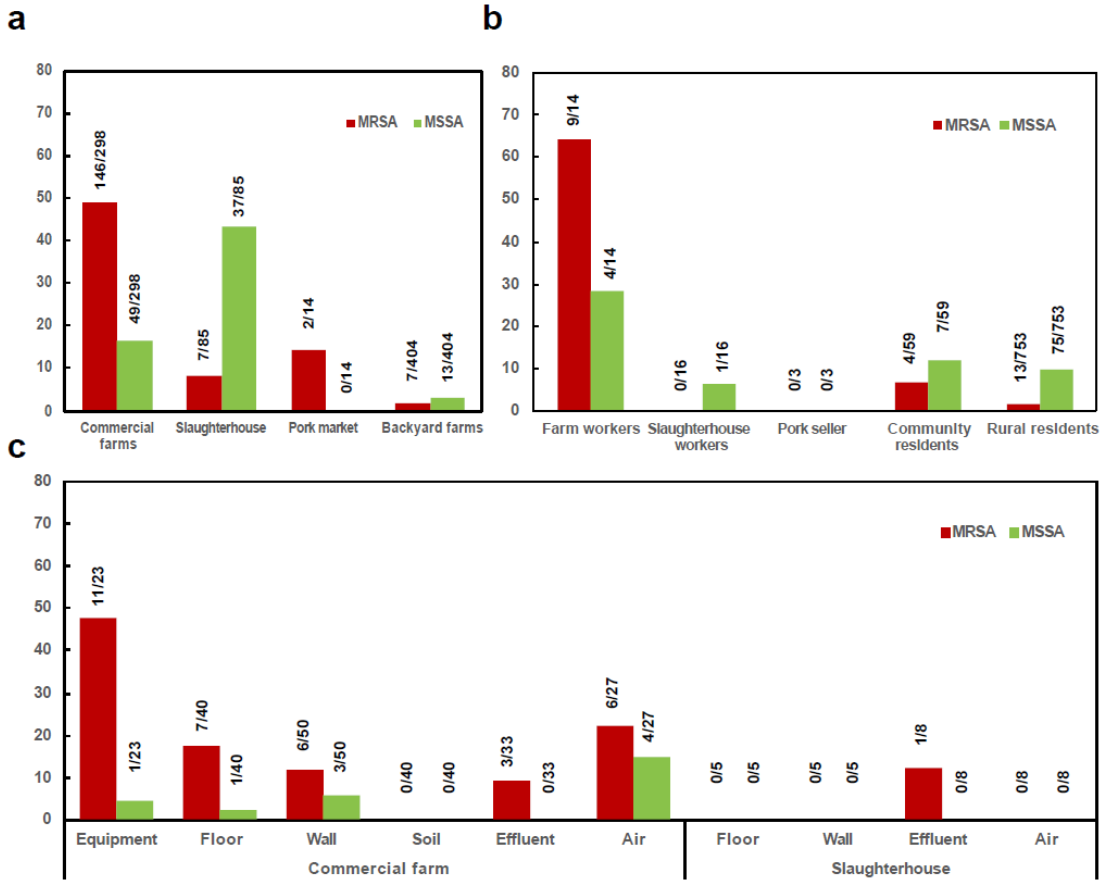

Figure 1. Prevalence of methicillin-sensitive *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* in samples of various origin along and around a pork production chain in Shandong Province, China. (a) Pig samples. (b) Human samples. (c) Environmental samples

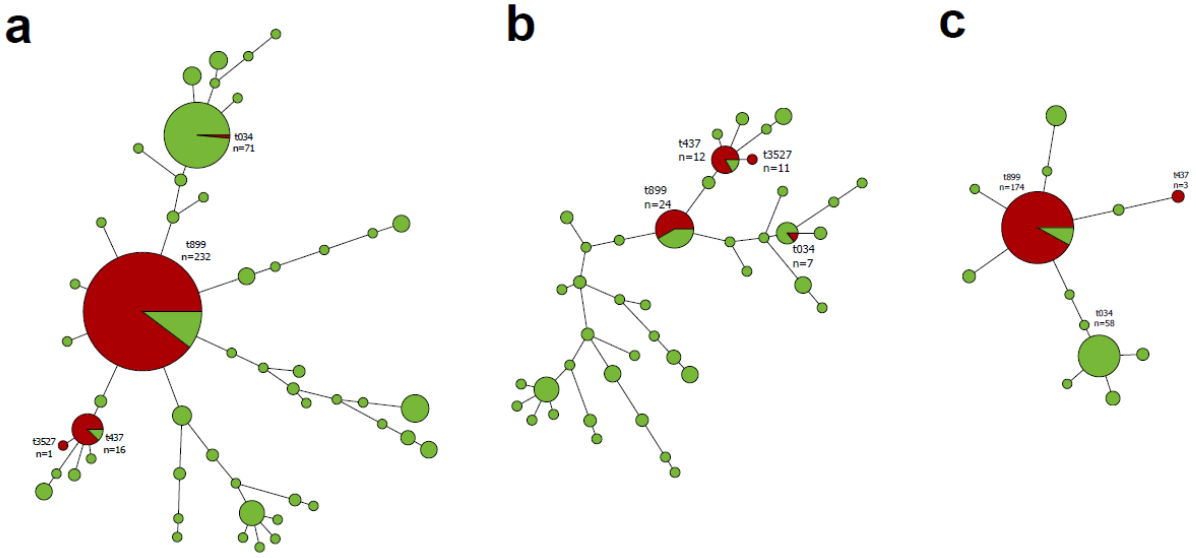

Figure 2. Minimum spanning tree of methicillin-sensitive *Staphylococcus aureus* (n=199) and methicillin-resistant *Staphylococcus aureus* (n=223) isolates by *spa-type*. (a) All isolates. (b) Isolates of human origin. (c) Isolates of pig origin. Each node represents a single *spa-type*. The size of the node is proportional to the number of isolates represented by a said node. Branch lengths between nodes are proportional to the number of alleles that differ between the two linked nodes. Selected nodes are labelled with corresponding *spa-type* and number of isolates represented. Red nodes, MRSA; Greed nodes, MSSA.

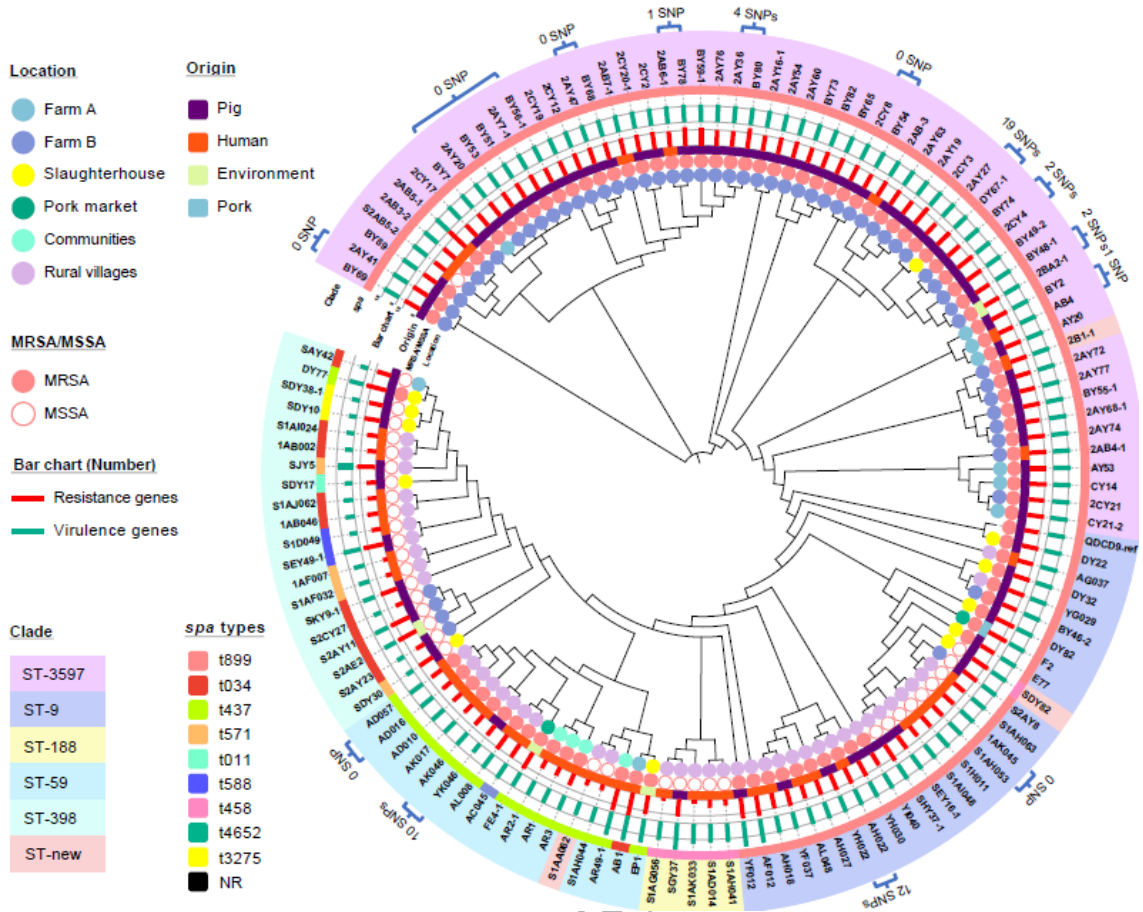

Figure 3. Genomic analysis of methicillin-sensitive *Staphylococcus aureus* (n = 46) and methicillin-resistant *Staphylococcus aureus* (n = 91) isolates of various origins along and around the pork production chain. A maximum-likelihood phylogenetic tree was constructed using the core genome SNPs. Sources of the isolates are indicated by different colours for origin (squares) and samples' location (circles). MRSA and MSSA are denoted by filled and empty pink circles, respectively. MLST and *spa-type*s depicted in different colours for strain identification (ID) and squares, respectively. The total number of antibiotic resistance genes (red) and virulence genes (green) in each isolate are denoted by the length of a bar chart. Details of the genes of each isolate are given in Table S2. For the convenience of discrimination, this core genome-based ML tree was visualised ignoring branch lengths.

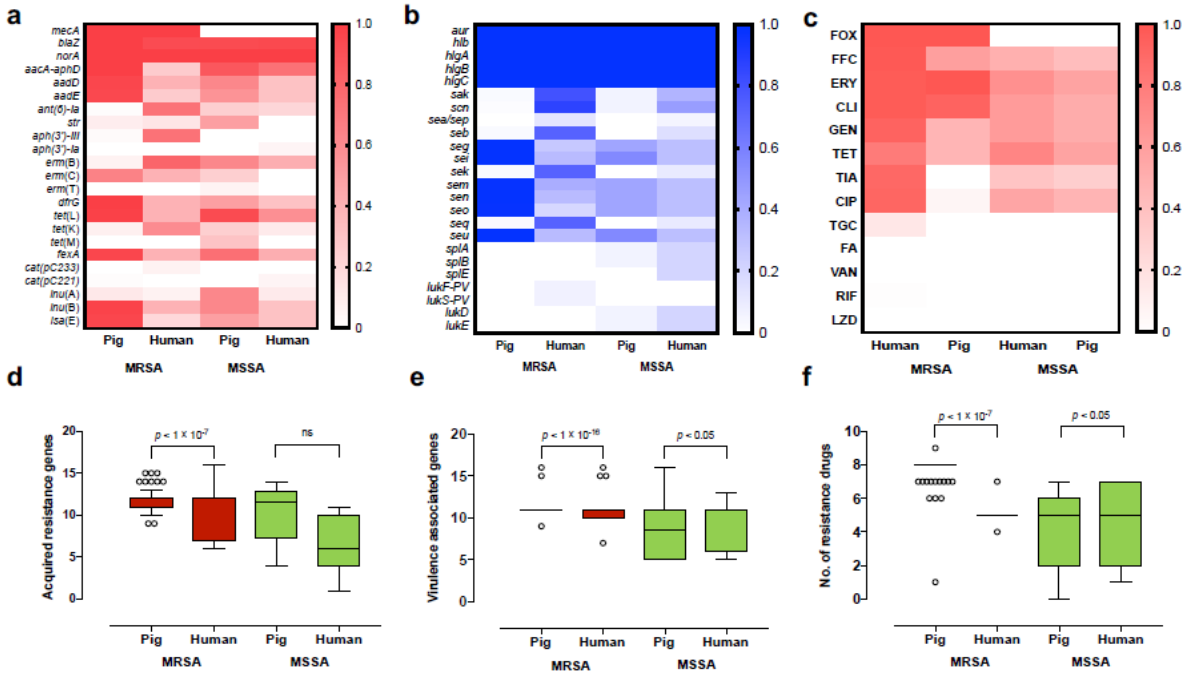

Figure 4. Heat map and prevalence of resistance genes, virulence genes and number of drugs with resistance of human- and pig-associated methicillin-resistant *Staphylococcus aureus* and methicillin-sensitive *Staphylococcus aureus* isolates. Each cell in the heat map indicates the percentage of strains containing particular resistance genes (a), virulence genes (b) or resistance to a particular drug (c). The bar graph indicates the total number of resistance genes (d), virulence genes (e) and the number of drugs with resistance (f) within each isolate of human- and pig-associated. Pig-associated MRSA (CC9, 76/77; ST398, 1/77), Human-associated MRSA (ST59, 15/15), Pig-associated MSSA (ST398, 11/16; ST9, 3/16; ST5053, 1/16, ST188, 1/16), Human-associated MSSA (ST398, 7/19; CC9, 6/19; ST188, 4/19; ST5052, 1/19; ST59, 1/19). Abbreviations: FOX, cefoxitin; FFC, florfenicol; ERY, erythromycin; CLI, clindamycin; GEN, gentamicin; TET, tetracycline; TIA, tiamulin; CIP, ciprofloxacin, TGC, tigecycline; FA, fusidic acid; VAN, Vancomycin; RIF, rifampicin; LZD, linezolid.