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

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ABSTRACT

Livestock-associated meticillin-resistant *Staphylococcus aureus* (LA-MRSA) is an increasingly important public health concern worldwide; however, data on LA-MRSA from Asian countries is scarce. As such, a comprehensive molecular epidemiological survey of *S. aureus* along a pork production chain and in the community was undertaken in Shandong Province, China. *spa* typing and whole-genome sequencing were used to survey the occurrence and potential transmission of *S. aureus* in various sectors, including 899 porcine 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 wholesale 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 wholesale market (pork, 14%; sellers, 0%) and 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, one MRSA ST398 was observed, and a novel CC9 variant ST3597 was detected within the chain. The high MRSA carriage rates and the emergence of a new MRSA CC9 variant identified in this study highlight the need for MRSA surveillance.

1. Introduction

The global epidemic of livestock-associated meticillin-resistant *Staphylococcus aureus* (LA-MRSA) among pigs has become a recognized public health concern [1–4]. In China, the occurrence and epidemiology of LA-MRSA have not been studied extensively compared with European countries. One major difference noted in China is that the LA-MRSA identified mainly belong to clonal complex (CC) 9 [4], whereas LA-MRSA CC398 dominates in Europe [5]. CC9 isolates were first reported in pigs and people with occupational contact with pigs in 2008 in China [6,7], but only a limited number of studies have described CC9 from livestock or slaughterhouses in China [4,8]. 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

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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 pork production, its environment and surrounding community in China.

To improve knowledge about the LA-MRSA situation in China, a cross-sectional study investigating both MRSA and meticillin-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, *spa* typing, whole-genome sequencing (WGS) and bioinformatic analysis were performed.

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 [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 1500 and 3000 pigs, respectively; a slaughterhouse, processing 1000–1500 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 from the slaughterhouse; Community 2, ~1.5 km from Farm B; and Community 3, ~2.0 km from Farm A. Each community consisted of ~150 households and, based on an estimated detection rate of 10% (from local Centre for Disease Control and Prevention), 40 random households were contacted. In the end, ~20 households per community participated. A nasal swab was collected from one adult volunteer from each household (Fig. S1, see online supplementary material).

For pig snout and human nasal swabs, the ESwab system (Copan, Brescia, Italy) was used according to the manufacturer's instructions. Snout swabs were collected randomly from sows (n=137), weaners (n=91) and growers (n=70) from the farms with one to three 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 (farms, 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 and Fig. S1, see online supplementary material).

Human and porcine samples from a previous study located in 12 rural villages neighbouring the production chain (slaughterhouse to the nearest and furthest 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 and Fig. S1, see online supplementary material) [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 *S. aureus* (CHROMagar Co.), respectively. One single putative MRSA and *S. aureus* colony was selected from the respective plate and subcultured 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 (Bruker, Bremen, Germany), with the suspected MRSA confirmed by polymerase chain reaction [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 documents VET08 [14] and M100-S28 [15]. *S. aureus* ATCC 29213 was used as the 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 *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 February 2017) [20] and VirulenceFinder 1.6 (18 February 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 visualized 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, La Jolla, CA, USA), and statistical analyses were performed in SPSS 25.0 (IBM Corp., Armonk, NY, 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 1983 samples collected from various sources and locations (Table S1, see online supplementary material), 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 (Fig. 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, see online supplementary material). 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) (Fig. 1b and Table S1, see online supplementary material) [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 Farms A and B, respectively. Most environmental samples from the slaughterhouse tested negative for both MRSA and MSSA, except for one MRSA-positive effluent sample (Fig. 1c and Table S1, see online supplementary material).

3.2. Population structure of MRSA and MSSA

In total, 51 *spa* types were identified among the 422 *S. aureus* isolates, with MSSA isolates (47 *spa* types) showing greater diversity than MRSA (four *spa* types) (Fig. 2a and Table S1, see online supplementary material). 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 (Fig. 2a).

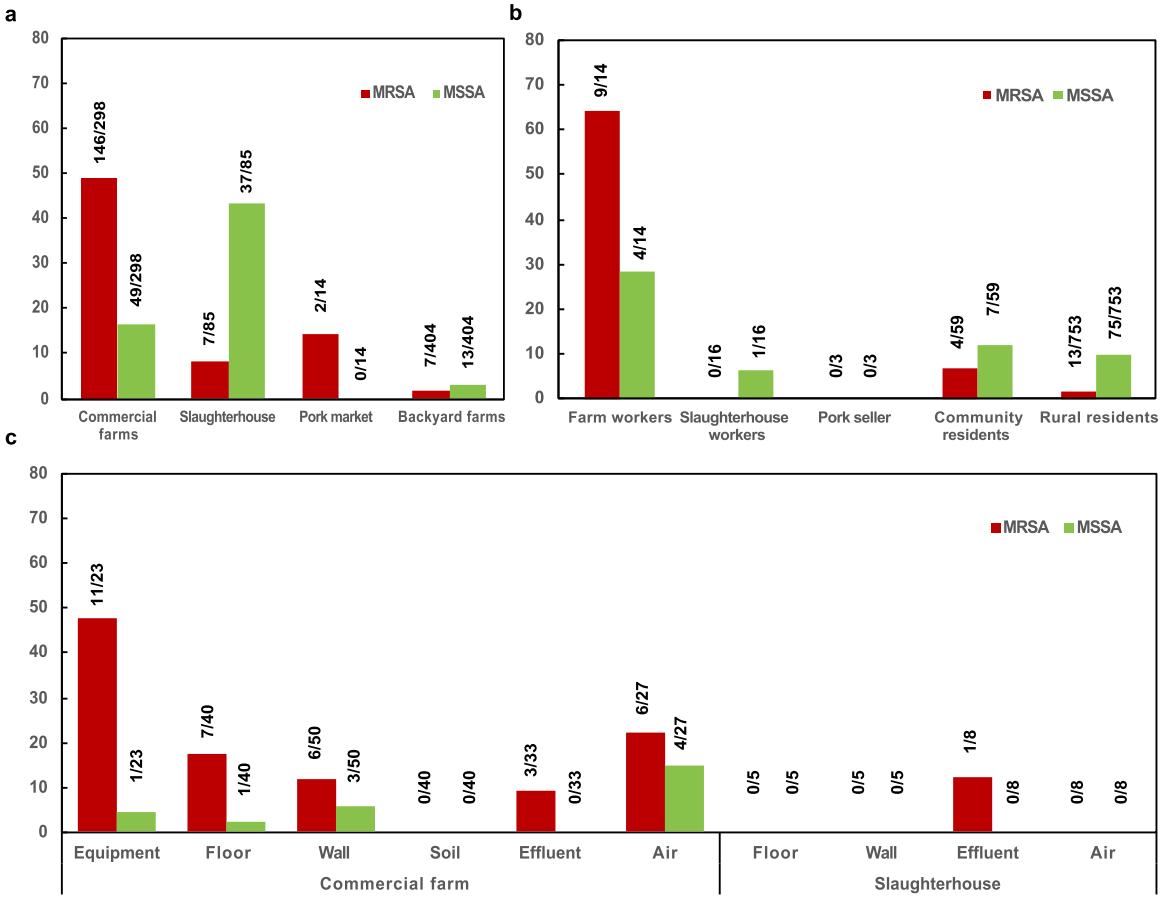

Fig. 1. Prevalence of meticillin-susceptible *Staphylococcus aureus* (MSSA) and meticillin-resistant *S. aureus* (MRSA) in samples of various origin along and around a pork production chain in Shandong Province, China. (a) Porcine samples. (b) Human samples. (c) Environmental samples.

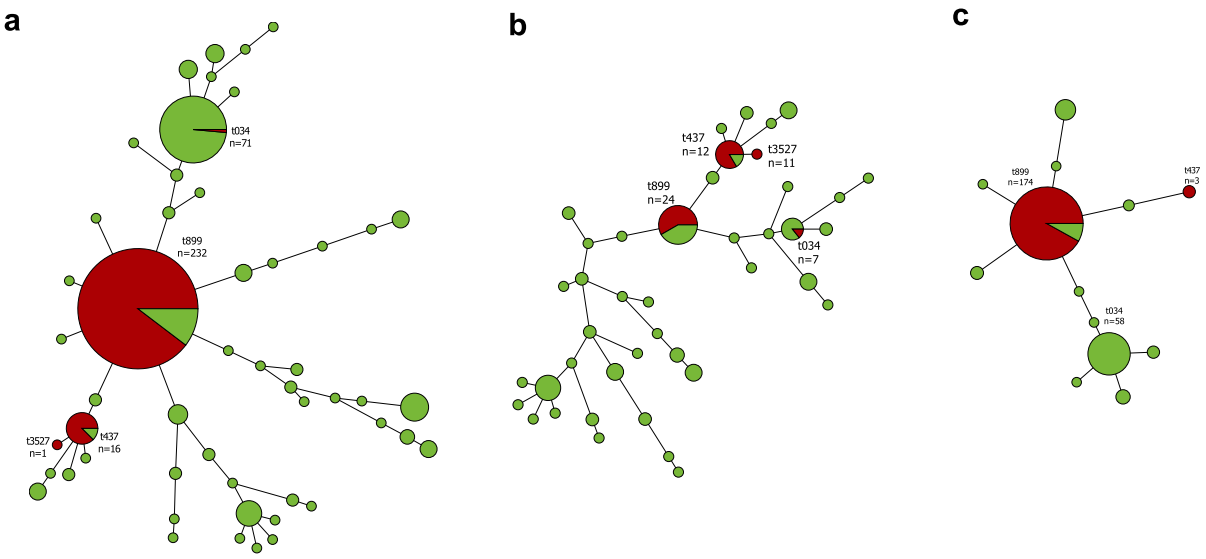

Fig. 2. Minimum spanning tree of meticillin-susceptible *Staphylococcus aureus* (MSSA, *n*=199) and meticillin-resistant *Staphylococcus aureus* (MRSA, *n*=223) isolates by *spa* type. (a) All isolates. (b) Isolates of human origin. (c) Isolates of porcine origin. Each node represents a single *spa* 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; green nodes, MSSA.

MRSA isolates of human origin were predominantly t899 (54%, 14/26) and t437 (39%, 10/26) (Fig. 2b), while the clear majority of porcine MRSA isolates were t899 (98%, 159/162) (Fig. 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 (Fig. 2b), while the 99 porcine MSSA isolates belonged to 12 *spa* types (Fig. 2c).

In total, 128 isolates – 92 MRSA belonging to four *spa* types and 36 MSSA belonging to eight *spa* types – were subjected to WGS (Fig. S2a and Table S2, see online supplementary material). 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 sequence types: one MRSA isolate, a single locus variant of ST3597; and two MSSA isolates, single locus variants of ST9 and ST59, respectively. These were assigned as ST5051, ST5052 and ST5053, respectively.

MRSA ST3597-t899 isolates were frequently identified in samples collected from commercial farms, including isolates 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, the slaughterhouse, the pork wholesale market and the rural villages were predominantly of the same MLST type, ST398, but representing a distinct set of *spa* types (Table S2, see online supplementary material).

Core-genome-based ML phylogenetic analysis of the 92 MRSA and the 36 MSSA isolates identified four groups, corresponding to sequence types identified as CC9, ST188, ST59 and ST398 (Fig. 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 (one SNP difference). This was also observed on Farm B, as ST3597-t899 MRSA 2AB6-1 (farm worker) and BY78 (weaner) were closely related (one 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) (zero to four SNPs difference), and ST3597-t899 MRSA isolates BY54 and BY74 recovered from weaners were closely related to isolates recovered from growers (2CY8 and 2CY4) (zero to two SNPs difference). Further, ST3597-t899 MRSA isolate 2BA2-1 recovered from an air sample on Farm B was closely related to the porcine isolate BY48-1 (one SNP difference) of the same farm. In addition, ST3597-t899 MRSA isolate BY7 recovered from a weaner on Farm A was identical to isolates recovered from sows (2AY7-1 and 2AY20) on Farm B (zero SNP divergence) (Fig. 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 (zero SNP difference), S1AH011 (ST9-t899) and S1AH053 (ST9-t899) from Village H (zero SNP difference) (Fig. 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 (Fig. 4a and b). Acquired AMR genes were widespread in isolates of both human and porcine 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

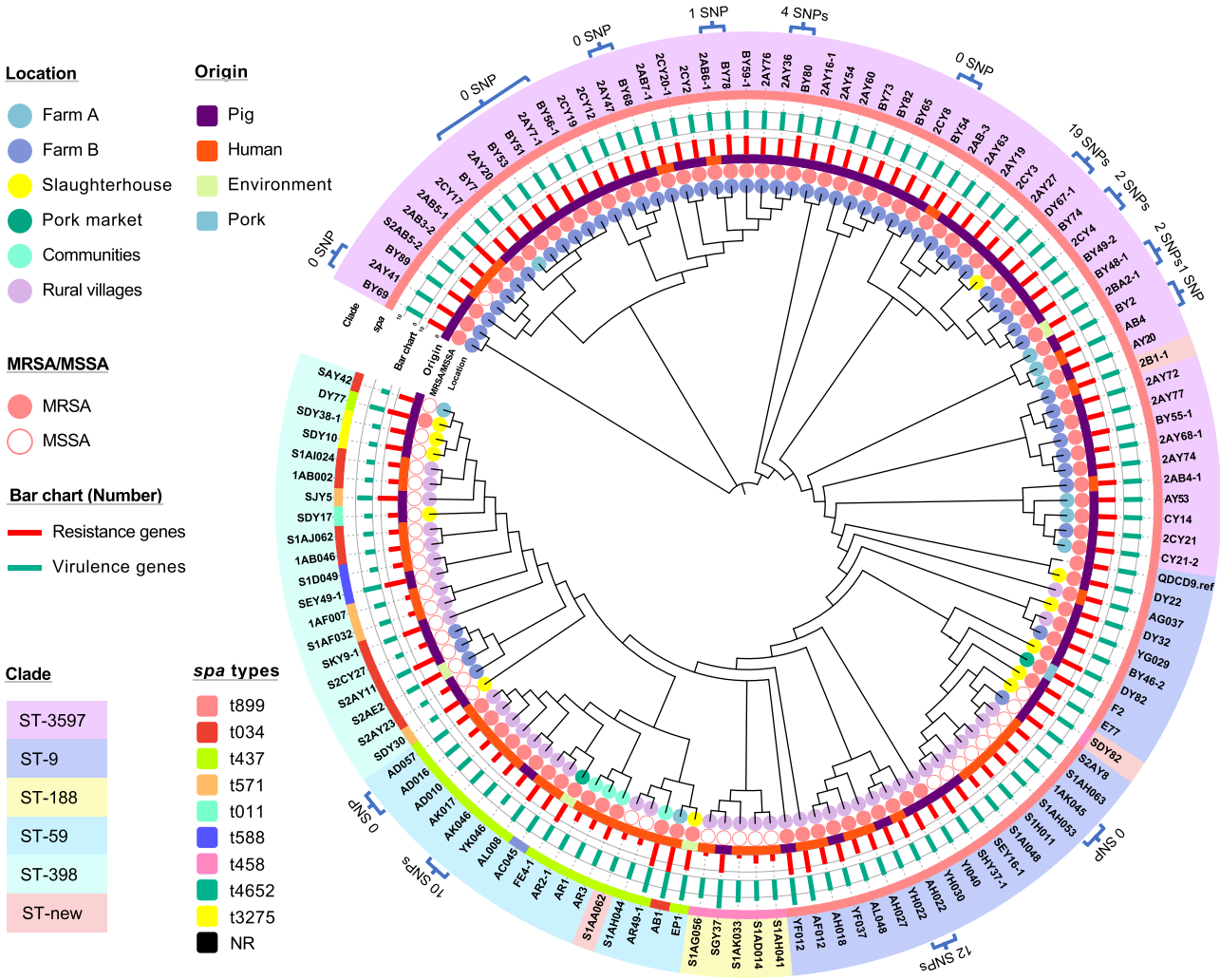

Fig. 3. Genomic analysis of meticillin-susceptible *Staphylococcus aureus* (MSSA, n=46) and meticillin-resistant *S. aureus* (n=91) isolates of various origins along and around the pork production chain. A maximum-likelihood phylogenetic tree was constructed using core-genome single nucleotide polymorphisms (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. Multi-locus sequence typing and *spa* types are 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 (see online supplementary material). For the convenience of discrimination, this core-genome-based maximum-likelihood tree was visualized ignoring branch lengths.

in pig-associated isolates than human-associated isolates (median 12 vs. 7; $P<1\times10^{-7}$) (Fig. 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; $P<1\times10^{-7}$) (Fig. 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; $P<1\times10^{-9}$) (Fig. 4a). Similarly, the tetracycline resistance gene *tet*(L) was strongly associated with MRSA of porcine 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; $P<1\times10^{-4}$) (Fig. 4a). The same was also observed for macrolide resistance genes. The gene *erm*(C) was associated with porcine 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 ($P<1\times10^{-8}$) (Fig. 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 porcine origin than human isolates (>96% of pig-associated isolates vs. ≤40% of human-associated isolates; $P<1\times10^{-7}$). Of the MSSA isolates of porcine origin, the median number of AMR genes was 11.5 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 human isolates ($P<0.05$), while no specific gene was associated with MSSA isolates of human origin (Fig. 4a).

Differences in virulence genes in each isolate were also observed between isolates of pig- and human-associated groups (MRSA, median 11 vs. 10, $P<1\times10^{-16}$; MSSA, median 8.5 vs. 11, $P<0.05$) (Fig. 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; $P<1\times10^{-7}$) (Fig. 4b). Only *sak* and *scn* were associated with MSSA

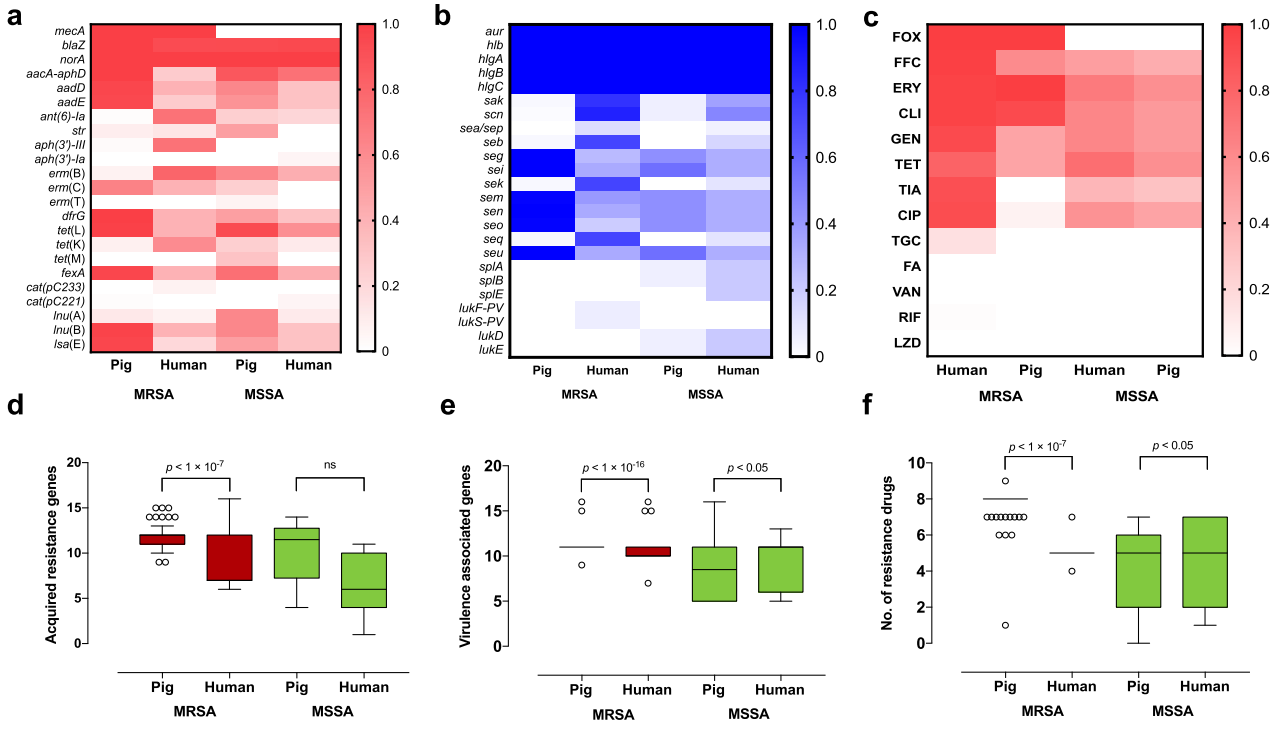

Fig. 4. Heat map and prevalence of resistance genes, virulence genes and number of drugs with resistance of human- and pig-associated meticillin-resistant *Staphylococcus aureus* (MRSA) and meticillin-susceptible *S. aureus* (MSSA) isolates. Each cell in the heat map indicates the percentage of strains 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 drugs with resistance (f) within each human- and pig-associated isolate. 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). 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.

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 porcine origin) recovered from rural villages were positive for *lukD* and *lukE*, with the *luk* genes coding for leucocidins. The isolates of porcine origin were all negative for Panton–Valentine leucocidin genes (Fig. 4b).

3.4. Antimicrobial susceptibility profiles of MRSA and MSSA

Compared with MRSA isolates from humans, MRSA isolates from pigs were resistant to a larger number of antimicrobial agents (median 8 vs. 5; $P<1\times10^{-7}$) (Fig. 4f). Resistance to florfenicol, gentamicin, tetracycline, tiamulin and ciprofloxacin were associated with MRSA isolates from pigs (81% of pig-associated isolates vs. ≤60% of human-associated isolates; $P<0.01$) (Fig. 4c). Erythromycin and clindamycin resistance were commonly observed in MRSA isolates regardless of their origin (human, 100%, 93%; pig, 97%, 97%) (Fig. 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 (Fig. 4c).

4. Discussion

To the authors' 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, MRSA-CC9 was detected from commercial farms, the slaughterhouse and the pork wholesale 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 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 which showed that the general population without livestock contact are less likely to carry MRSA-CC9 [28]. The high occurrence in pig production is still of concern because increased incidence of LA-MRSA-CC398 has been observed among humans without livestock contact in Europe [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 size of Farm B [29]. This observation was not surprising as a previous study in Shandong Province described great variations in 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 were ST3597-t899, a single locus variant of ST9. ST3597 were widely distributed in the two commercial farms among pigs, farm workers and environmental samples, and occurred sporadically at the slaughterhouse (Fig. S2b, see online supplementary material). 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 (Fig. S2b, see online supplementary material). These results indicate a recent clonal expansion of ST3597 in Farms A and B, rather than multiple introductions into these farms. To the authors' 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, ST398 is primarily associated with community- and hospital-acquired MSSA infections in humans [27,31]. Similar to a previous finding [32], four of seven ST398-MSSA isolated from humans carried genes related to the immune evasion gene cluster (IEC), while none of the 12 isolates from pigs carried these genes (Table S2, see online supplementary material). This indicates that two different ST398 populations are circulating in the area, one in humans and another 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 *spa* type t437, which was associated with ST59 in this and other studies [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 *spa* types t034 and t571 [34].

The occurrence of resistance genes in MRSA and MSSA porcine 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 concordance between AMR genotypes and phenotypes for most of the isolates. The differences in AMR between human and porcine 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 enterotoxin 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 this study.

This study provides 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 the environment within the commercial farms, between pigs in commercial farms and the slaughterhouse, and 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, this study found two separate MRSA populations in this region of China: 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). In addition, 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 and further research studies in pigs, humans and their environment are needed.

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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).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.03.022.

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