Veterinary Microbiology 201 (2017) 183–187

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Short communication

Characterization of pig-associated methicillin-resistant *Staphylococcus aureus*

Jun Li[a,b], Nansong Jiang[a], Yuebin Ke[c], Andrea T. Feßler[b], Yang Wang[a], Stefan Schwarz[a,b,d,*], Congming Wu[a,**]

[a] Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Veterinary Medicine, China Agricultural University, Beijing 100193, PR China
[b] Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut (FLI), Neustadt-Mariensee, Germany
[c] Key Laboratory of Genetics & Molecular Medicine of Shenzhen, Shenzhen Center for Disease Control and Prevention, Shenzhen 518055, PR China
[d] Institute of Microbiology and Epizootics, Department of Veterinary Medicine, Freie Universität Berlin, Robert-von-Ostertag-Straße 7-13, 14163 Berlin, Germany

ARTICLE INFO

Article history:
Received 27 November 2016
Received in revised form 30 December 2016
Accepted 1 January 2017

Keywords:
LA-MRSA
Pig
Multi-resistance
Molecular typing

ABSTRACT

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) have been reported in various countries worldwide. However, although China is one of the biggest pig and pork producers, large-scale studies on pig-associated LA-MRSA from China are scarce. The aims of this study were to analyze 2420 non-duplicate samples collected from pigs at swine farms and slaughterhouses in different regions in China during 2014 for the prevalence of pig-associated MRSA and to determine the antimicrobial resistance pheno- and genotypes of the respective isolates. MRSA isolates were identified in 270 (11.2%) samples. The isolates were characterized by antimicrobial susceptibility testing, multilocus sequence typing (MLST), *spa* typing, pulsed-field gel electrophoresis (PFGE) and screening for resistance genes. All MRSA isolates belonged to the clonal complex 9 and *spa* type t899, but showed variable PFGE patterns. All isolates were non-susceptible to oxacillin, cefoxitin, clindamycin, chloramphenicol, florfenicol, ciprofloxacin, and valnemulin. High rates of resistance were also observed for tetracycline (99.6%), erythromycin (97.0%), quinupristin-dalfopristin (97.0%), and gentamicin (80.4%). Three linezolid-non-susceptible isolates containing the multi-resistance gene *cfr* and nine rifampicin-non-susceptible isolates with mutations in *rpoB* were detected. Resistance to β-lactams was exclusively associated with *mecA*, while phenicol resistance was mainly attributable to *fexA*, except in the three *cfr*-positive isolates. The pleuromutilin-lincosamide-streptogramin A resistance gene *lsa*(E) was identified in all MRSA isolates, and no other pleuromutilin resistance genes, except *cfr* in three isolates, were detected. Pigs are the most important hosts of LA-MRSA in China. Screening for pig-associated MRSA is necessary to monitor changes in epidemiology and characteristics of these important pathogens.

1. Introduction

Staphylococcus aureus is a commensal opportunistic pathogen present in both healthy and diseased humans and animals. Methicillin-resistant *S. aureus* (MRSA) is considered to be the most important cause of nosocomial and community-acquired infections worldwide (Woodford and Livermore, 2009). MRSA evolved from methicillin-susceptible *S. aureus* by acquisition of *mecA*. Livestock-associated MRSA (LA-MRSA) was first detected in 2004 in the Netherlands and France, and has since gained much attention (Armand-Lefevre et al., 2005; Voss et al., 2005). The prevalence and spread of LA-MRSA differs geographically. The predominant clonal complex (CC) in Europe and USA is CC398, with most strains belonging to sequence type (ST) 398, whereas in Asia, CC9 strains are most common with ST9 being the most prevalent sequence type (Chuang and Huang, 2015).

Further studies of LA-MRSA showed that it was prevalent in a wide range of animal species, particularly in pigs (Weese, 2010; Wendlandt et al., 2013a). Human infections caused by pig-

* Corresponding author at: Institute of Microbiology and Epizootics, Department of Veterinary Medicine, Freie Universität Berlin, Robert-von-Ostertag-Straße 7-13, 14163 Berlin, Germany.
** Corresponding author at: Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Veterinary Medicine, China Agricultural University, Beijing 100193, PR China.
E-mail addresses: stefan.schwarz@u-berlin.de (S. Schwarz), wucm@cau.edu.cn (C. Wu).

http://dx.doi.org/10.1016/j.vetmic.2017.01.017
0378-1135/

associated MRSA indicated that pigs are an important reservoir for MRSA, and that these bacteria are transferred to people via occupational exposure to pigs (Fitzgerald, 2012). Thus, the potential risk of transmission of LA-MRSA between animals and humans is a growing health concern. For this reason, ongoing surveillance is necessary for combating the further dissemination of LA-MRSA.

To date, there have been few reports regarding pig-associated MRSA in mainland China. In 2008, dust samples from nine farrow-to-finish pig farms in Sichuan Province were screened for MRSA, and samples from five of the nine farms were MRSA-positive (Wagenaar et al., 2009). In the same year, the first study on the prevalence of MRSA colonization of pigs in mainland China was conducted, and reported a LA-MRSA isolation rate in pigs of 11.4% (Cui et al., 2009). In 2009, nasal swabs were collected from 590 pigs in two slaughterhouses in northeastern China, and 38 (6.4%) samples were MRSA-positive. All MRSA isolates in this study were identified as ST9 (Yan et al., 2014). MRSA from pigs has also been reported in Hong Kong, which imports pigs from mainland China, with prevalence varying between 16 and 39.3% (Guardabassi et al., 2009; Ho et al., 2012a,b). In these cases, all pig-associated MRSA strains belonged to CC9 and showed *spa* type t899.

The aims of the present study were: (i) to investigate the prevalence of MRSA colonization of pigs in mainland China in 2014, (ii) to examine the phenotype and genotype of antimicrobial resistance in pig-associated MRSA isolates, and (iii) to determine the molecular characteristics of pig-associated MRSA.

2. Materials and methods

2.1. Sample collection

During June and July 2014, a total of 2420 non-duplicate nasal swabs were collected from pig farms and slaughterhouses in Shanghai (n = 251, samples from two slaughterhouses), Henan (n = 870, samples from one pig farm and one slaughterhouse), Ningxia (n = 473, samples from one slaughterhouse and two pig farms), and Shandong (n = 826, samples from 25 pig farms), China. The pig farms and slaughterhouses were selected at random from the intensive pig farms or slaughterhouses in each region, but were widely dispersed geographically. Farm samples were collected from finishing pigs of different herds, while samples from slaughterhouses were collected immediately after slaughter from individual pigs. For each slaughterhouse, samples were collected over the course of a single day. All nasal swabs were placed into tubes containing 1 ml of 7.5% sodium chloride broth, which contained 0.5% beef powder, 7.5% sodium chloride and 1.0% peptone, before being transported to the laboratory.

2.2. MRSA isolation and identification

The sodium chloride broths containing the swabs were incubated at 37 °C for 24 h. Following incubation, cultures were plated onto *S. aureus* selective agar plates (CHROMagar Staph aureus, Becton Dickinson GmbH, Paris, France) supplemented with 4 mg/L cefoxitin. Following incubation for 24 h at 37 °C, any pink/mauve colonies were selected as suspected *S. aureus* isolates. Further identification was performed by multiplex-PCR analysis, as described previously (Louie et al., 2002).

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using both the broth microdilution and disk diffusion methods according to documents VET01-S4 and M100-S25 of the Clinical and Laboratory Standards Institute (CLSI, 2015a,b). The following antimicrobial agents were tested: oxacillin, cefoxitin, clindamycin, erythromycin, chloramphenicol, florfenicol, linezolid, and vancomycin for broth microdilution, and rifampicin (5 μg), gentamicin (10 μg), quinupristin/dalfopristin (15 μg), tetracycline (30 μg), and ciprofloxacin (5 μg), all obtained from Oxoid (Basingstoke, UK), for disk diffusion. To examine pleuromutilin resistance, all pig-associated MRSA isolates identified in this study were screened on brain heart infusion agar (AOBOX, Beijing, China) plates containing 1 mg/L valnemulin.

2.4. Resistance gene detection

In addition to the β-lactam resistance genes *mecA* and *mecC*, all pig-associated MRSA isolates were also screened by PCR for the presence of the following resistance genes: *fexA*, *fexB*, *cfr*, *optrA*, *lsa*(E), *lsa*(B), *vga*(A), *vga*(B), *vga*(C), *vga*(D), *vga*(E), *vga*(E)$_v$, and *vga*(A)$_{LC}$ (Ho et al., 2012a; Wendlandt et al., 2015a,b; Li et al., 2016) accounting for resistance to phenicols, oxazolidinones, pleuromutilins, lincosamides, and/or streptogramin A antibiotics.

2.5. Molecular typing

Pulsed-field gel electrophoresis (PFGE) with SmaI was used to characterize all isolates. An 80% cutoff was used to define a PFGE cluster. One or two isolates from each PFGE pattern were selected from each region of China for use as representative isolates for multilocus sequence typing (MLST) and *spa* typing. MLST was conducted by amplifying and sequencing fragments of seven housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*) (Enright et al., 2000). The MLST type was determined by comparison with the sequences deposited in the online MLST database (http://saureus.beta.mlst.net/). The *spa* typing was performed by amplifying and sequencing the variable repeat region of the *spa* gene, which was then compared with the *spa* sequences of the respective online database (http://spa.ridom.de/) (Harmsen et al., 2003).

3. Results

3.1. Prevalence of pig-associated MRSA

MRSA was identified in 270 (11.2%) of the 2420 samples. The prevalence of MRSA differed significantly between the different regions: Shanghai (46.6%), Henan (5.2%), Ningxia (3.6%), and Shandong (12.0%). MRSA isolates were identified at a particularly high rate from both of the slaughterhouses in Shanghai, and were present in samples from 14/25 farms in Shandong. However, isolation rates were much lower in samples from Henan and Ningxia provinces (Table S1).

3.2. Antimicrobial susceptibility testing

All 270 pig-associated MRSA isolates were non-susceptible to oxacillin, cefoxitin, clindamycin, chloramphenicol, florfenicol, and ciprofloxacin. Moreover, all of the isolates grew on selective plates containing 1 mg/L valnemulin. High resistance rates were also observed for tetracycline (99.6%), erythromycin (97.0%), quinupristin-dalfopristin (97.0%), and gentamicin (80.4%). Nine rifampicin-non-susceptible MRSA isolates (two from Shanghai and seven from Shandong) were identified. Two isolates from Shanghai and one from Shandong were non-susceptible to linezolid. All of the isolates were susceptible to vancomycin (Table 1).

Table 1
Antimicrobial resistance profiles of pig-associated methicillin-resistant *Staphylococcus aureus* isolates from different regions.

Antimicrobial category	Antimicrobial agent	Shanghai (n = 113)		Henan (n = 45)		Ningxia (n = 17)		Shandong (n = 95)	
		NS	S	NS	S	NS	S	NS	S
β-lactams	Oxacillin	113	0	45	0	17	0	95	0
	Cefoxitin	113	0	45	0	17	0	95	0
Macrolides	Erythromycin	112	1	45	0	17	0	88	7
Lincosamides	Clindamycin	113	0	45	0	17	0	95	0
Phenicols	Chloramphenicol	113	0	45	0	17	0	95	0
	Florfenicol	113	0	45	0	17	0	95	0
Tetracyclines	Tetracycline	112	1	45	0	17	0	95	0
Fluoroquinolones	Ciprofloxacin	113	0	45	0	17	0	95	0
Streptogramins	Quinupristin-dalfopristin	112	1	45	0	17	0	88	7
Aminoglycosides	Gentamicin	77	36	30	15	15	2	95	0
Ansamycins	Rifampicin	2	111	0	45	0	17	7	88
Glycopeptides	Vancomycin	0	113	0	45	0	17	0	95
Oxazolidinones	Linezolid	2	111	0	45	0	17	1	94

NS = non-susceptible (intermediate + resistant); S = susceptible.

3.3. Resistance gene detection

The presence of genes conferring resistance to β-lactams, phenicols, oxazolidinones, pleuromutilins, lincosamides, and/or streptogramin A antibiotics was confirmed by PCR. Most of the resistance genes were homogeneously distributed among all isolates. Resistance to β-lactams was exclusively associated with the presence of *mecA*; no *mecC* carriers were identified. The pleuromutilin-lincosamide-streptogramin A resistance gene *lsa*(E) was identified in all of the MRSA isolates, and no other pleuromutilin resistance genes, except *cfr* in three isolates, were detected. Three linezolid-non-susceptible isolates contained the multiresistance gene *cfr*, which confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A. Most of the phenicol-resistant isolates, except the three *cfr*-positive isolates, harbored *fexA*. None of the isolates contained the florfenicol resistance gene *fexB*, or the novel oxazolidinone-phenicol resistance gene *optrA*.

3.4. Molecular typing

The 270 isolates were assigned to 11 PFGE types (A–K). PFGE types A (40.7%), C (19.6%), and D (10.7%) accounted for 71.0% of the isolates. Two PFGE patterns, types B and D, were detected in all four geographic regions, while PFGE patterns H, I, and K, each with only one or two isolates, were more unevenly distributed (Table 2). Based on the PFGE typing results, 44 isolates belonging to different PFGE patterns and originating from each of the four regions were selected as representative isolates for MLST and *spa* analyses. Except one isolate from Henan, which was ST1376-t899, all of the other representative isolates were ST9-t899 (Fig. 1). ST1376 is a single locus variant of ST9 (allele 73 instead of allele 3 in *aroE*) and also belongs to CC9.

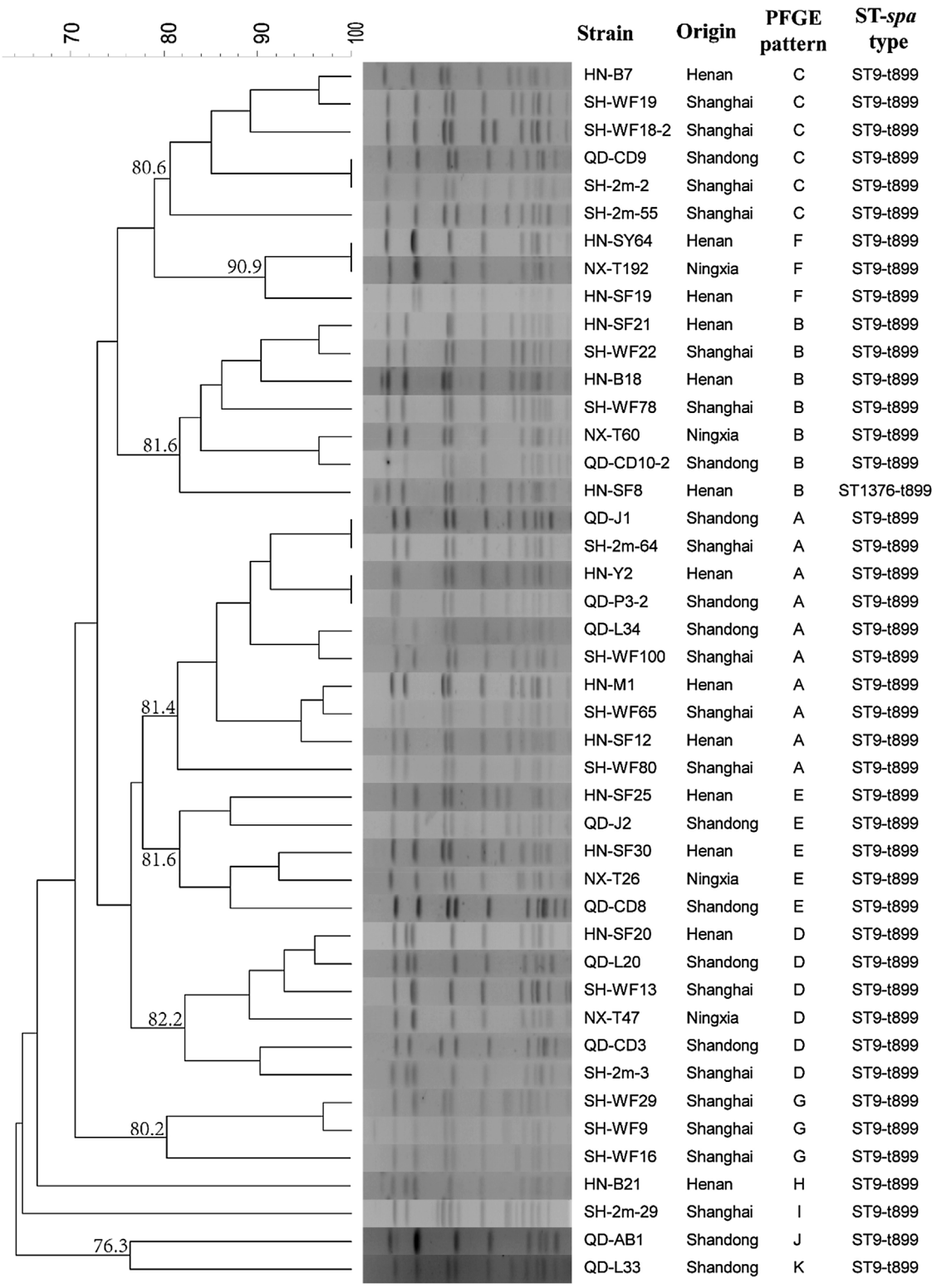
4. Discussion

This study investigated pig-associated MRSA using a large number of samples collected from four different geographical areas in China. The results indicated a higher average prevalence of nasal MRSA carriage of pigs in China (11.2%) compared with other Asian countries, including Japan (0.9%, 1/115 samples) (Baba et al., 2010), Malaysia (1.4%, 5/360 samples) (Neela et al., 2009), and South Korea (3.2%, 21/657 samples) (Lim et al., 2012). Possible explanations for the increased prevalence include differences in the isolation methods, livestock density, antimicrobial usage, and management systems between China and other Asian countries. However, compared with previous studies in China, the average prevalence of nasal carriage of pig-associated MRSA in this study was similar to that described in a study conducted in 2008 (11.4%), in which samples were also collected from both pig farms and slaughterhouses (Cui et al., 2009). This observation suggested that the average nasal carriage rate of MRSA was rather stable during recent years in China. However, differences were seen between the MRSA prevalence in the different regions investigated with highest prevalence being detected in the two slaughterhouses from Shanghai. It has been shown that pigs can become colonized with MRSA in the short period of time between transportation from the farm to the slaughterhouse (Broens et al., 2011). A longitudinal study showed that pigs from a MRSA-negative farm became MRSA-positive after co-transportation with MRSA-positive pigs (Bangerter et al., 2016). This may explain the higher prevalence of MRSA at the slaughterhouses in Shanghai in this study as well as in another study in which samples were only collected from one slaughterhouse (Ho et al., 2012b). Compared with other regions, pigs from Ningxia had a much lower prevalence of MRSA carriage. A possible reason for this could be the lower livestock density and the different dietary habits in this region.

All of the pig-associated MRSA isolates in this study were multidrug-resistant. Antimicrobial agents are used in the rearing of food-producing animals not only for prevention and treatment of disease, but also for growth promotion. Based on the records of antibiotic usage in commercial farms, antimicrobial agents, including enrofloxacin, tetracycline, oxytetracycline, chlortetracycline, lincomycin, and tiamulin, are commonly used in pig farming in China. The use of these antibiotics may influence the antibiotic resistance patterns of pig-associated MRSA. Our previous study showed that rifampicin-non-susceptibility of the isolates from Shanghai and Shandong was caused by mutations in the gene *rpoB*. These mutations may occur spontaneously or originate from co-selection in the presence of fluoroquinolones (Li et al., 2016). Unexpectedly, all of the pig-associated MRSA isolates identified in this study carried the multiresistance gene *lsa*(E) which confers resistance to lincosamides, pleuromutilins and streptogramin A antibiotics. The location of *lsa*(E) on a plasmid may play a role in its persistence and dissemination (Li et al., 2013; Wendlandt et al.,

Table 2
Pulsed-field gel electrophoresis patterns of pig-associated methicillin-resistant *Staphylococcus aureus* isolates collected from different regions.

region	PFGE pattern										
	A	B	C	D	E	F	J	H	I	G	K
Shanghai	43	14	23	17	0	6	0	0	1	9	0
Henan	12	5	18	1	4	4	0	1	0	0	0
Ningxia	0	1	0	1	2	13	0	0	0	0	0
Shandong	55	5	12	10	6	0	5	0	0	0	2
total no.	110	25	53	29	12	23	5	1	1	9	2



Strain	Origin	PFGE pattern	ST-*spa* type
HN-B7	Henan	C	ST9-t899
SH-WF19	Shanghai	C	ST9-t899
SH-WF18-2	Shanghai	C	ST9-t899
QD-CD9	Shandong	C	ST9-t899
SH-2m-2	Shanghai	C	ST9-t899
SH-2m-55	Shanghai	C	ST9-t899
HN-SY64	Henan	F	ST9-t899
NX-T192	Ningxia	F	ST9-t899
HN-SF19	Henan	F	ST9-t899
HN-SF21	Henan	B	ST9-t899
SH-WF22	Shanghai	B	ST9-t899
HN-B18	Henan	B	ST9-t899
SH-WF78	Shanghai	B	ST9-t899
NX-T60	Ningxia	B	ST9-t899
QD-CD10-2	Shandong	B	ST9-t899
HN-SF8	Henan	B	ST1376-t899
QD-J1	Shandong	A	ST9-t899
SH-2m-64	Shanghai	A	ST9-t899
HN-Y2	Henan	A	ST9-t899
QD-P3-2	Shandong	A	ST9-t899
QD-L34	Shandong	A	ST9-t899
SH-WF100	Shanghai	A	ST9-t899
HN-M1	Henan	A	ST9-t899
SH-WF65	Shanghai	A	ST9-t899
HN-SF12	Henan	A	ST9-t899
SH-WF80	Shanghai	A	ST9-t899
HN-SF25	Henan	E	ST9-t899
QD-J2	Shandong	E	ST9-t899
HN-SF30	Henan	E	ST9-t899
NX-T26	Ningxia	E	ST9-t899
QD-CD8	Shandong	E	ST9-t899
HN-SF20	Henan	D	ST9-t899
QD-L20	Shandong	D	ST9-t899
SH-WF13	Shanghai	D	ST9-t899
NX-T47	Ningxia	D	ST9-t899
QD-CD3	Shandong	D	ST9-t899
SH-2m-3	Shanghai	D	ST9-t899
SH-WF29	Shanghai	G	ST9-t899
SH-WF9	Shanghai	G	ST9-t899
SH-WF16	Shanghai	G	ST9-t899
HN-B21	Henan	H	ST9-t899
SH-2m-29	Shanghai	I	ST9-t899
QD-AB1	Shandong	J	ST9-t899
QD-L33	Shandong	K	ST9-t899

Fig. 1. PFGE patterns and ST-*spa* types of representative isolates selected from different regions. 80% cutoff was used to define a PFGE cluster.

2013b, 2015b). Linezolid-non-susceptible isolates containing the phenicol-lincosamide-oxazolidinone-pleuromutilin-streptogramin A resistance gene *cfr* were detected in two different regions. Although clindamycin and linezolid are not approved for use in pig farming, selective pressure from other antibiotics, such as lincomycin, florfenicol and tiamulin, that are widely used in food-producing animals could promote the spread of *cfr*. In addition, the emergence and dissemination of these multidrug-resistant isolates in pigs pose a significant risk to people with continuous exposure to livestock.

As shown by the PFGE typing results, each region had its own predominant PFGE pattern. The appearance of MRSA isolates with the same PFGE pattern in different regions implied an exchange of MRSA strains between the regions, most likely through the transfer

of animals. MLST and *spa* typing were used as basic typing tools for tracing the evolutionary origin and spread of MRSA, but their discriminatory power appeared too limited for pig-associated MRSA in China. Although the PFGE patterns of the isolates showed more variation than was observed by MLST and *spa* typing, new technologies, such as next generation sequencing, may provide better options for understanding the origin, transmission and evolution of LA-MRSA. Therefore, these advanced technologies will be included in our further studies of the origin and spread of pig-associated MRSA in China.

5. Conclusion

In the present study conducted in 2014, the nasal carriage rate of MRSA in pigs (11.2%) was similar compared to that reported by Cui and co-workers in 2008 (11.4%) (Cui et al., 2009). ST9-t899 was still the predominant clonal type of LA-MRSA isolates in China, and the pig-associated MRSA isolates identified in this study showed resistance to a wide range of antibiotics. Significantly, all of the isolates carried the multi-resistance gene *lsa*(E), with three isolates also harboring the multi-resistance gene *cfr*. As zoonotic infections caused by MRSA ST9 have been reported in the Netherlands (van Loo et al., 2007) and in China (Liu et al., 2009), ongoing surveillance is needed to detect changes in the characteristics and epidemiology of these strains.

Funding

This study was financially supported by the grants from the National Natural Science Foundation of China (31472237 and 31370046), and the German Federal Ministry of Education and Research (BMBF) through the German Aerospace Center (DLR), grant number 01KI1301D (MedVet-Staph 2).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vetmic.2017.01.017.

References

Armand-Lefevre, L., Ruimy, R., Andremont, A., 2005. Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs. Emerg. Infect. Dis. 11, 711–714.

Baba, K., Ishihara, K., Ozawa, M., Tamura, Y., Asai, T., 2010. Isolation of meticillin-resistant *Staphylococcus aureus* (MRSA) from swine in Japan. Int. J. Antimicrob. Agents 36, 352–354.

Bangerter, P.D., Sidler, X., Perreten, V., Overesch, G., 2016. Longitudinal study on the colonisation and transmission of methicillin-resistant *Staphylococcus aureus* in pig farms. Vet. Microbiol. 183, 125–134.

Broens, E.M., Graat, E.A., Van der Wolf, P.J., Van de Giessen, A.W., De Jong, M.C., 2011. Transmission of methicillin resistant *Staphylococcus aureus* among pigs during transportation from farm to abattoir. Vet. J. 189, 302–305.

Clinical and Laboratory Strandards Institute, 2015a. Performance Standands for Antimicrobial Susceptibility Testing; Twenty-Fifth Information Supplement M100-S25. Clinical and Laboratory Strandards Institute, Wayne, PA.

Clinical and Laboratory Strandards Institute, 2015b. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals. CLSI Supplement VET01-S, 3rd ed. Clinical and Laboratory Strandards Institute, Wayne, PA.

Chuang, Y.Y., Huang, Y.C., 2015. Livestock-associated meticillin-resistant *Staphylococcus aureus* in Asia: an emerging issue? Int. J. Antimicrob. Agents 45, 334–340.

Cui, S., Li, J., Hu, C., Jin, S., Li, F., Guo, Y., Ran, L., Ma, Y., 2009. Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from swine and workers in China. J. Antimicrob. Chemother. 64, 680–683.

Enright, M.C., Day, N.P., Davies, C.E., Peacock, S.J., Spratt, B.G., 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J. Clin. Microbiol. 38, 1008–1015.

Fitzgerald, J.R., 2012. Livestock-associated *Staphylococcus aureus*: origin, evolution and public health threat. Trends Microbiol. 20, 192–198.

Guardabassi, L., O'Donoghue, M., Moodley, A., Ho, J., Boost, M., 2009. Novel lineage of methicillin-resistant *Staphylococcus aureus*, Hong Kong. Emerg. Infect. Dis. 15, 1998–2000.

Harmsen, D., Claus, H., Witte, W., Rothganger, J., Claus, H., Turnwald, D., Vogel, U., 2003. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. J. Clin. Microbiol. 41, 5442–5448.

Ho, J., O'Donoghue, M., Guardabassi, L., Moodley, A., Boost, M., 2012a. Characterization of methicillin-resistant *Staphylococcus aureus* isolates from pig carcasses in Hong Kong. Zoonoses Publ. Health 59, 416–423.

Ho, P.L., Chow, K.H., Lai, E.L., Law, P.Y., Chan, P.Y., Ho, A.Y., Ng, T.K., Yam, W.C., 2012b. Clonality and antimicrobial susceptibility of *Staphylococcus aureus* and methicillin-resistant *S. aureus* isolates from food animals and other animals. J. Clin. Microbiol. 50, 3735–3737.

Li, B., Wendlandt, S., Yao, J., Liu, Y., Zhang, Q., Shi, Z., Wei, J., Shao, D., Schwarz, S., Wang, S., Ma, Z., 2013. Detection and new genetic environment of the pleuromutilin-lincosamide-streptogramin A resistance gene *lsa*(E) in methicillin-resistant *Staphylococcus aureus* of swine origin. J. Antimicrob. Chemother. 68, 1251–1255.

Li, J., Feßler, A., Jiang, N., Fan, R., Wang, Y., Wu, C., Shen, J., Schwarz, S., 2016. Molecular basis of rifampicin resistance in multi-resistant porcine livestock-associated methicillin-resistant *Staphylococcus aureus*. J. Antimicrob. Chemother 71, 3313–3315.

Lim, S.K., Nam, H.M., Jang, G.C., Lee, H.S., Jung, S.C., Kwak, H.S., 2012. The first detection of methicillin-resistant *Staphylococcus aureus* ST398 in pigs in Korea. Vet. Microbiol. 155, 88–92.

Liu, Y., Wang, H., Du, N., Shen, E., Chen, H., Niu, J., Ye, H., Chen, M., 2009. Molecular evidence for spread of two major methicillin-resistant *Staphylococcus aureus* clones with a unique geographic distribution in Chinese hospitals. Antimicrob. Agents Chemother. 53, 512–518.

Louie, L., Goodfellow, J., Mathieu, P., Glatt, A., Louie, M., Simor, A.E., 2002. Rapid detection of methicillin-resistant staphylococci from blood culture bottles by using a multiplex PCR assay. J. Clin. Microbiol. 40, 2786–2790.

Neela, V., Mohd Zafrul, A., Mariana, N.S., van Belkum, A., Liew, Y.K., Rad, E.G., 2009. Prevalence of ST9 methicillin-resistant *Staphylococcus aureus* among pigs and pig handlers in Malaysia. J. Clin. Microbiol. 47, 4138–4140.

van Loo, I., Huijsdens, X., Tiemersma, E., de Neeling, A., van de Sande-Bruinsma, N., Beaujean, D., Voss, A., Kluytmans, J., 2007. Emergence of methicillin-resistant *Staphylococcus aureus* of animal origin in humans. Emerg. Infect. Dis. 13, 1834–1839.

Voss, A., Loeffen, F., Bakker, J., Klaassen, C., Wulf, M., 2005. Methicillin-resistant *Staphylococcus aureus* in pig farming. Emerg. Infect. Dis. 11, 1965–1966.

Wagenaar, J.A., Yue, H., Pritchard, J., Broekhuizen-Stins, M., Huijsdens, X., Mevius, D. J., Bosch, T., Van Duijkeren, E., 2009. Unexpected sequence types in livestock associated methicillin-resistant *Staphylococcus aureus* (MRSA): MRSA ST9 and a single locus variant of ST9 in pig farming in China. Vet. Microbiol. 139, 405–409.

Weese, J.S., 2010. Methicillin-resistant *Staphylococcus aureus* in animals. ILAR J. 51, 233–244.

Wendlandt, S., Feßler, A.T., Monecke, S., Ehricht, R., Schwarz, S., Kadlec, K., 2013a. The diversity of antimicrobial resistance genes among staphylococci of animal origin. Int. J. Med. Microbiol. 303, 338–349.

Wendlandt, S., Lozano, C., Kadlec, K., Gomez-Sanz, E., Zarazaga, M., Torres, C., Schwarz, S., 2013b. The enterococcal ABC transporter gene *lsa*(E) confers combined resistance to lincosamides, pleuromutilins and streptogramin A antibiotics in methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. J. Antimicrob. Chemother. 68, 473–475.

Wendlandt, S., Kadlec, K., Feßler, A.T., Schwarz, S., 2015a. Identification of ABC transporter genes conferring combined pleuromutilin-lincosamide-streptogramin A resistance in bovine methicillin-resistant *Staphylococcus aureus* and coagulase-negative staphylococci. Vet. Microbiol. 177, 353–358.

Wendlandt, S., Shen, J., Kadlec, K., Wang, Y., Li, B., Zhang, W.J., Feßler, A.T., Wu, C., Schwarz, S., 2015b. Multidrug resistance genes in staphylococci from animals that confer resistance to critically and highly important antimicrobial agents in human medicine. Trends Microbiol. 23, 44–54.

Woodford, N., Livermore, D.M., 2009. Infections caused by Gram-positive bacteria: a review of the global challenge. J. Infect. 59 (Suppl. 1), S4–S16.

Yan, X., Yu, X., Tao, X., Zhang, J., Zhang, B., Dong, R., Xue, C., Grundmann, H., Zhang, J., 2014. *Staphylococcus aureus* ST398 from slaughter pigs in northeast China. Int. J. Med. Microbiol. 304, 379–383.