International Journal of Medical Microbiology 304 (2014) 379–383

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

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

Staphylococcus aureus ST398 from slaughter pigs in northeast China

Xiaomei Yan[a,1], Xiaojie Yu[b,1], Xiaoxia Tao[a], Jianfeng Zhang[b], Binghua Zhang[a], Rui Dong[b], Chengyu Xue[b], Hajo Grundmann[c,**], Jianzhong Zhang[a,*]

[a] *State Key Laboratory for Infectious Disease Prevention and Control, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China*
[b] *Heilongjiang Province Center for Disease Control and Prevention, Harbin, China*
[c] *Department of Medical Microbiology, University Medical Center Groningen and University of Groningen, The Netherlands*

ARTICLE INFO

Article history:
Received 14 August 2013
Received in revised form
22 November 2013
Accepted 15 December 2013

Keywords:
Staphylococcus aureus
Molecular epidemiology
Antimicrobial susceptibility
Livestock
ST398

ABSTRACT

To describe the prevalence and population structure of *Staphylococcus aureus* bacteria that colonize pigs at slaughterhouses in northeastern China, nose swabs were collected from pigs in two slaughterhouses in Harbin, Heilongjiang Province, China in 2009. *S. aureus* isolates were characterized by multilocus sequence typing (MLST), *spa* typing, SCC*mec* typing, antimicrobial susceptibility testing and *pvl* gene detection. A total of 200 *S. aureus* isolates were collected from 590 pigs (33.9%, 200/590), of which 162 (81%, 162/200) were methicillin-susceptible *S. aureus* (MSSA) and 38 (19%, 38/200) were methicillin-resistant *S. aureus* (MRSA). Ninety-nine of the MSSA isolates (99/162, 61.1%) were ST398, which represented the dominant sequence type overall. Eighty-seven isolates were ST9 (87/200, 43.5%), and all MRSA belonged to that sequence type which consisted of the *spa* types t899 and t2922. Among the MSSA strains, t034, t899 and t4358 were the most dominant *spa* types (139/162, 85.8%). All MRSA isolates harbored SCC*mec* type IVb. The *pvl* gene was only detected in 3 ST7/t2119 MSSA isolates. All MRSA but more importantly also 82.7% (134/162) of the MSSA isolates were resistant to six or more antibiotics. Moreover, a novel resistance determinant-*lsa(E)* was identified among 22% (44/200) of all isolates. In conclusion, pigs in northeast China are frequently colonized with ST398 MSSA. MRSA with this sequence type, typically associated with pigs in Europe, was not found. High levels of multiple antibiotic resistance among MRSA isolates as well as MSSA isolates are a public health concern.

Introduction

Staphylococcus aureus is a well-known commensal and pathogen of many animal species, including humans. Although most *S. aureus* are host-specific, the potential for animals to act as a source of *S. aureus* infections for humans has been shown for some clonal lineages, such as sequence type (ST) 398 (Garcia-Alvarez et al., 2011; Van Cleef et al., 2011). Moreover, the propensity of *S. aureus* to develop multiple drug resistance (MDR) has played the key role in triggering pandemic spread (Holden et al., 2013). Hence, farm animals could be an important ecological niche for the emergence of MDR *S. aureus* because massive antibiotics use for treatment, prevention of diseases or growth promotion provides the necessary evolutionary constraints.

Methicillin-resistant *S. aureus* (MRSA) in pigs was first reported in France and was subsequently demonstrated to belong to a distinct clone, ST398 (Armand-Lefevre et al., 2005). Later, the ST398 MRSA clone was discovered to be widespread in pig farms in the Netherlands, where transmission to humans was reported for the first time (Voss et al., 2005; Armand-Lefevre et al., 2005). ST398 MRSA has been widely identified in several countries in Europe (Witte et al., 2007), North America (Smith et al., 2009; Khanna et al., 2008) and Asia (Lim et al., 2012; Sergio et al., 2007). ST398 MRSA possess several typical features, including (i) non-typability by standard *SmaI* pulsed-field gel electrophoresis analysis due to DNA methylation, (ii) t034, t011, and t108 being the dominant *spa* types, (iii) presence of SCC*mec* types IVa or V, (iv) absence of *luk*S and *luk*F coding for PVL and (v) resistance to tetracycline (Vanderhaeghen et al., 2010; Bens et al., 2006). Persons in direct contact with MRSA-positive animals have an increased risk of becoming MRSA positive (Morgan, 2008).

In China, previous studies revealed ST9 MRSA isolates as a predominant clone in pigs (Wagenaar et al., 2009; Cui et al., 2009), whereas ST398-MRSA-V-*spa* t034 was only reported once, in a pet hospital in Beijing (Zhang et al., 2011), and was likely of human origin. Little is known about the prevalence, population structure and

* Corresponding author at: 155 Changbai Road, Changping District, Beijing 102206, China. Tel.: +86 10 58900707; fax: +86 10 58900790.
** Corresponding author at: Hanzeplein 1, 9713 GZ Groningen, The Netherlands. Tel.: +31 50 3619156; fax: +31 50 3619156.
E-mail addresses: h.grundmann@umcg.nl (H. Grundmann), zhangjianzhong@icdc.cn (J. Zhang).
[1] These authors contributed equally to this work.

1438-4221/$ – see front matter © 2014 Published by Elsevier GmbH.
http://dx.doi.org/10.1016/j.ijmm.2013.12.003

antibiotic susceptibility of methicillin-susceptible *S. aureus* (MSSA) carried by pigs in China. The objective of this study was to characterize the prevalence of *S. aureus* and its population structure among colonized pigs in northeastern China.

Materials and methods

Sample collection

From September through November 2009, nose swabs were taken from 590 pigs in two slaughterhouses located 68 km apart in Harbin city, Heilongjiang Province, northeastern China. Pigs came to one slaughterhouse from 26 medium-sized industrial farms and to the other slaughterhouse from 29 small private farms. Pigs were slaughtered at the same day after they were collected from different farms. Samples from individual pigs were processed separately.

Sample preparation

Swabs were enriched in tryptic soy broth (OXOID, Basingstoke, England) with 7% NaCl at 37 °C for 24 h and then plated onto mannitol salt agar (OXOID, Basingstoke, UK) and cultured at 37 °C for 24 h. Presumptive *S. aureus* colonies were identified at the species level by coagulase production using the Slidex Staph Plus kit (Murex Biotech, Kent, UK) and PCR for the *nuc* gene (Brakstad et al., 1992). The presence of the *mecA* gene was determined and used to define the MRSA genotype (Brakstad et al., 1992).

Molecular typing

All of the isolates were characterized using *spa* typing (Harmsen et al., 2003) and multilocus sequence typing (MLST) (Enright et al., 2000). Based Upon Repeat Pattern (BURP) analysis was used to group *spa* types into *spa* clonal complexes (*spa*-CCs) (Mellmann et al., 2007). The *spa* type was excluded from BURP analysis if it was shorter than 5 repeats. All MRSA isolates were investigated by SCC*mec* typing (Zhang et al., 2005).

Antimicrobial susceptibility

A total of 21 antimicrobial agents were tested, including penicillin (PEN), cefoxitin (FOX), tetracycline (TET), chlortetracycline (CTT), ciprofloxacin (CIP), chloramphenicol (CHL), erythromycin (ERY), clindamycin (CLI), gentamicin (GEN), rifampicin (RIF), trimethoprim-sulfamethoxazole (SXT), nitrofurantoin (NIT), quinupristin/dalfopristin (Q/D), vancomycin (VAN), teicoplanin (TEC), linezolid (LZD), tigecycline (TGC), daptomycin (DAP), fusidic acid (FUS), fosfomycin (FOF) and mupirocin (MUP). Eighteen antimicrobial agents were tested using the agar dilution method on Mueller-Hinton agar; the others (quinupristin/dalfopristin, tigecycline and daptomycin) were tested by Etest (AB bioMérieux, Solna, Sweden). The MICs for most of the antimicrobials were interpreted using CLSI criteria (Clinical and Laboratory Standards Institute, 2010), but the EUCAST clinical breakpoint (www.eucast.org) was used for fosfomycin and chlortetracycline. The resistance breakpoints for mupirocin and fusidic acid were defined as previously described (Finlay et al., 1997; MacGowan and Wise, 2001). *S. aureus* ATCC29213 was included for internal quality control. All quinupristin/dalfopristin resistant isolates were investigated for the resistance genes *vat*(A), *vat*(B), *vat*(C), *vga*(A), *vga*(A)*variant*, *vga*(B), *vga*(C), *vga*(E), *cfr* and *lsa*(E) by PCR (Table S1).

pvl gene detection

Panton-Valentine leukocidin (PVL) genes (*luk*S-PV and *luk*F-PV) were detected by PCR as previously described (Lina et al., 1999).

Statistical analysis

The quantitative variables were analyzed using chi-square tests in PASW Statistics 18.0.3 (SPSS Inc., Chicago, Illinois, US). P-values of ≤ 0.05 were considered statistically significant.

Results

Prevalence of S. aureus

A total of 200 *S. aureus* isolates were recovered from 590 slaughter pigs (200/590, 33.9%), including 38 MRSA isolates (38/590, 6.4%). The prevalence of MRSA among pigs and the number of MRSA-positive farms were significantly higher in Slaughterhouse 1 (Table 1).

Molecular characterization of S. aureus isolates

MLST typing for all the isolates revealed seven ST types: ST7, ST9, ST398, ST2113, ST2366, ST1375 and ST2773 (Table 2). Among MSSA, ST398 was the most frequent ST type and was identified in 99 of 162 isolates (99/162, 61.1%); this was followed by ST9 (49/162, 30.2%). ST9 was the only ST type among the 38 MRSA isolates. ST2113, ST2366 and ST2773 were new ST types. ST2366 represents a single locus variant (SLV) of ST9, while ST1375 represents a SLV of ST398. *Spa* typing of all of the isolates identified nine *spa* types and 2 *spa*-CCs (Table 2). The dominant *spa* type was t899 (33/38, 86.8%) among MRSA isolates, while t034 and t899 were the most prevalent *spa* types among MSSA, representing 55.6% (90/162) and 30.9% (50/162), respectively. Based on *spa* repeat and BURP analysis, two new *spa* types, t1446 and t5462, belonging to ST398 may have evolved in the MSSA isolates from t034 *spa* types that acquired one new *spa* repeat (r31) and three *spa* repeats (r34-r24-r25), respectively. In MRSA isolates, t899 is the probable ancestor of t4358 and t4474, which have separately acquired one *spa* repeat (r02) and changed one *spa* repeat from r07 to r26 by point mutation. All MRSA isolates were SCC*mec* type IVb and *pvl* negative. Three MSSA isolates with ST7 were found to be *pvl* positive.

Antimicrobial susceptibility

All MRSA isolates were resistant to most of the antibiotics tested (Table 3). Importantly, two predominant multidrug resistance profiles were identified: PEN-FOX-TET-CTT-CIP-CHL-ERY-CLI-GEN-QDA and PEN-FOX-TET-CTT-CIP-CHL-ERY-CLI-GEN-QDA-SXT, which accounted for 39.5% (15/38) and 36.8% (14/38) of all MRSA isolates, respectively. The majority of MSSA isolates (134/162, 82.7%) were resistant to six or more antibiotics. Compared with MRSA isolates, the resistance profiles of MSSA isolates were more diverse. Twenty-six antibiotic profiles were identified and PEN-TET-CTT-CIP-ERY-CLI-GEN-CHL, PEN-TET-CTT-CIP-ERY-CLI-GEN and PEN-TET-CIP-ERY-CLI-GEN accounted for 59.9% (97/162) of all resistance profiles in MSSA isolates. All MRSA and seven MSSA isolates were resistant to quinupristin/dalfopristin.

Furthermore, PCR analysis revealed that 44 of 45 quinupristin/dalfopristin resistant strains were positive for the *lsa*(E) gene. In contrast, the remaining 1 isolate harbored none of the currently known quinupristin/dalfopristin resistance genes. ST9 was the only ST type among the 44 *lsa(E)* positive strains of which 7 (7/44, 15.9%) were MSSA and 37 were MRSA(37/44, 84.1%)(Table S2). Two *spa* types were observed of which t899 was the dominant

Table 1
Prevalence of *Staphylococcus aureus* and Methicillin-resistant *S. aureus* (MRSA) among pigs from two slaughterhouses in northeast China during September–November 2009.

Slaughterhouse	No. of nasal swabs	No. of *S. aureus* (%)	No. of MRSA (%)	MRSA/*S. aureus* (%)	No. of farms with MRSA (%)
S1	313	104 (104/313, 33.2%)	31 (31/313, 9.9%)	29.8	14 (14/26, 53.8%)
S2	277	96 (96/277, 34.7%)	7 (7/277, 2.5%)	7.3	6 (6/29, 20.7%)
P-value	–	0.714	<0.0001	<0.0001	0.011

Table 2
Molecular characteristics of *Staphylococcus aureus* isolates from slaughter pigs.

MLST types	SPA types (CCs)	MSSA/MRSA	Number of isolates	SCC*mec*	Number of PVL$^+$ isolates
ST398	t034 (034)	MSSA	89	–	0
	t1446 (034)	MSSA	7	–	0
	t5462 (034)	MSSA	3	–	0
ST9	t899 (899)	MSSA	36	–	0
	t4358 (899)	MSSA	12	–	0
	t4474 (899)	MSSA	1	–	0
	t899 (899)	MRSA	33	IVb	0
	t2922 (excluded)	MRSA	4	IVb	0
	NT	MRSA	1	IVb	0
ST7	t2119 (singleton)	MSSA	8	–	3
ST2113	t6386 (singleton)	MSSA	2	–	0
ST1375	t034(034)	MSSA	1	–	0
ST2773	t899 (899)	MSSA	1	–	0
ST2366	NT	MSSA	1	–	0
Non-typable (NT)	NT	MSSA	1	–	0

type among 100% (7/7) *lsa(E)* positive MSSA and 86.5% (32/37) *lsa(E)* positive MRSA strains and t2922 corresponded to 10.8% (4/37) *lsa(E)* positive MRSA strains.

Discussion

Heilongjiang is an important agricultural province in China. In 2011, 16 million pigs were raised for food production in this province alone. Until now, no data have been available on the occurrence of MRSA and MSSA in pigs from this area. The prevalence of MRSA (38/590, 6.4%) in pigs observed in this study was lower than the prevalence in other regions in China (Cui et al., 2009; Wang et al., 2012), Europe (Tenhagen et al., 2009; de Neeling et al., 2007; Gomez-Sanz et al., 2010) and North America (Smith et al., 2009; Khanna et al., 2008), but higher than the prevalence in some other Asian countries (Lim et al., 2012; Neela et al., 2009; Baba et al., 2010). Despite this moderate frequency of MRSA among slaughter pigs in our study, multiple drug resistance (MDR) phenotype defined as resistance against 6 or more antibiotic was 86% (172/200) among all isolates suggestive of a significant reservoir of antibiotic resistance among farm animals in China.

Differences were observed in the prevalence of MRSA and the number of farms with MRSA between the two slaughterhouses. It can be speculated that the origins of these pigs and their rearing patterns were different. Slaughterhouse 1 was much bigger than slaughterhouse 2. Most of the pigs in slaughterhouse 1 came from larger farms, while in slaughterhouse 2 most of the pigs came from smaller farms where animals were kept free-range. Although MRSA carriage and transmission among pigs may not be a significant problem in this region, monitoring the occurrence of resistance in pigs may be advisable because certain clones may have the propensity to expand in the human population.

This is the first study to report the genetic population structure of *S. aureus* (MRSA and MSSA) from slaughterhouse pigs in

Table 3
Antimicrobial susceptibility of Methicillin-resistant *Staphylococcus aureus* (MRSA) and Methicillin-resistant *S. aureus* (MSSA) from slaughter pigs.

Antimicrobial agent	MRSA(38)				MSSA(162)			
	MIC range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%R	MIC range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%R
penicillin	2–128	64	128	100	0.064–8	2	4	99
cefoxitin	32–256	64	128	100	2–4	4	4	0
tetracycline	16–128	64	64	100	0.125–128	64	128	92
chlortetracycline	32–64	32	64	100	0.5–64	32	64	70
ciprofloxacin	1–64	16	32	97	0.5–64	32	>32	88
chloramphenicol	8–64	64	64	97	4–128	8	64	45
erythromycin	512	>256	>256	100	0.25–512	>256	>256	89
clindamycin	512	>256	>256	100	0.0625–512	>256	>256	88
gentamicin	16–64	32	64	100	0.125–128	16	64	79
rifampicin	0.002–8	0.016	8	16	0.002–4	0.004	0.06	1
trimethoprim/sulfamethoxazole	0.06–64	2	8	37	0.016–64	0.125	4	13
nitrofurantoin	16–512	32	>256	21	8–32	32	32	0
quinupristin/dalfopristin	4–8	4	8	100	0.25–4	0.5	2	4
vancomycin	0.5–2	1	1	0	0.5–2	1	1	0
teicoplanin	0.25–1	1	1	0	0.5–4	1	1	0
linezolid	1–4	2	2	0	0.75–4	2	4	0
tigecycline	0.38–0.5	0.5	0.5	0	0.094–0.5	0.19	0.38	0
daptomycin	0.19–0.5	0.25	0.38	0	0.094–0.38	0.125	0.19	0
fusidic acid	0.25–0.5	0.25	0.5	0	0.125–0.25	0.25	0.25	0
fosfomycin	4–16	8	16	0	1–16	4	8	0
mupirocin	0.125–2	0.125	0.25	0	0.125–38	0.25	0.5	0

northeast China. We found that ST398 was the most frequent ST type (99/162, 61.1%) among MSSA, whereas MRSA with this sequence type, typically associated with pigs in Europe, was not identified. ST398 MSSA is also a frequent community and hospital-acquired infections associated strain among humans in China (Zhao et al., 2012; Wu et al., 2010; Chen et al., 2010). In Europe, ST398 MRSA is associated with animal origin and is occasionally transmitted to humans via occupational exposure to farm animals. Whole-genome sequencing approaches and microarrays analysis of a large amount of ST398 isolates from different host species suggested that ST398 was most likely of human origin, and was occasionally transmitted to livestock companied with acquisition of SCC*mec* (Price LB et al., 2012; Uhlemann et al., 2012). Our study did not sample persons in direct contact with pigs, such as slaughterhouse workers and farmers. Whether transmission between humans and pigs is occurring in China is still unknown, but such transmission is likely.

Resistance to multiple antibiotics, not only among MRSA isolates, but notably also among MSSA isolates, is a matter of concern. It suggests that a substantial amount of antimicrobials are used in pig farming for growth promotion, prophylactic or therapeutic purposes. A high prevalence of multiple antimicrobial resistance in pig isolates was also found in another province (Shanxi) (Wang et al., 2012), and an abundance of diverse antibiotic resistance genes (ARGs) have been found on Chinese pig farms (Zhu et al., 2013); in contrast, most MSSA in Chinese hospitals have maintained a high degree of susceptibility to most antimicrobial agents, mainly exhibiting erythromycin and clindamycin resistance (Xiao et al., 2011). No doubt, there remains a potential threat to human health that resistant strains or resistance genes that have emerged in pigs (Zhu et al., 2013) are introduced into human health care institutions.

In this study, all quinupristin/dalfopristin resistant strains belonged to ST9. This may be the result of clonal expansion most likely originating from breeding farms and the selection of ST9 by the use of virginiamycin against which the quinupristin/dalfopristin resistant isolates exhibit cross-resistance or deficiency of DNA restriction systems likely to facilitate the horizontal gene transfer of "foreign" DNA, as has been found in ST398 (Schijffelen et al., 2010). Virginiamycin belongs to the same antibiotic group (streptogramin) as quinupristin/dalfopristin. This compound has been widely used for more than 25 years as an animal growth promoter (AGP) in poultry, cattle and swine. Because of concerns that cross resistance between virginiamycin and quinupristin/dalfopristin could lead to treatment failure in humans, virginiamycin was banned from animal use in Europe. In China, however, it is still widely used. In our study, 98% (44/45) of the quinupristin/dalfopristin resistant strains harbored the new resistance gene, *lsa(E)*, which was first described recently (Wendlandt et al., 2013; Li et al., 2013). High homology between the sequence of the new transposon carrying *lsa(E)* in *S. aureus* and the sequence of the *Enterococcus faecalis* plasmid pEF418 demonstrated the horizontal gene transfer interspecies (Wendlandt et al., 2013). So far, the gene *lsa(E)* has been only described in ST9 strains (both MRSA and MSSA) in China, but different *spa* types, antibiotic resistance profile and genomic location of the gene *lsa(E)* were observed. Whether the transposon has entered ST9 only once and then diversified and spread to different regions in China or, more likely, that the transposon prefers the ST9 background and has been acquired by ST9 on several occasions still need to be explained.

To our surprise, ST398 MSSA was the predominant clone among slaughterhouse pigs in northeast China. We hope that this finding will improve the understanding of the global population history of ST398.

Acknowledgements

This work was supported by the Major Infectious Diseases Such as AIDS and Viral Hepatitis Prevention and Control Technology Major Projects (2008ZX10004-002 and 2008ZX10004-008) of the Ministry of Science and Technology, People's Republic of China, by the State Key Laboratory for Infectious Disease Prevention and Control Fund (2012SKLID202), and by an educational grant from the Graduate School of Medical Sciences of the University of Groningen, The Netherlands.

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.ijmm.2013.12.003.

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.

Bens, C.C., Voss, A., Klaassen, C.H., 2006. Presence of a novel DNA methylation enzyme in methicillin-resistant *Staphylococcus aureus* isolates associated with pig farming leads to uninterpretable results in standard pulsed-field gel electrophoresis analysis. J. Clin. Microbiol. 44, 1875–1876.

Brakstad, O.G., Aasbakk, K., Maeland, J.A., 1992. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. J. Clin. Microbiol. 30, 1654–1660.

Chen, H., Liu, Y., Jiang, X., Chen, M., Wang, H., 2010. Rapid change of methicillin-resistant *Staphylococcus aureus* clones in a Chinese tertiary care hospital over a 15-year period. Antimicrob. Agents Chemother. 54, 1842–1847.

CLSI, 2010. Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement. CLSI document M100-S20. Clinical and Laboratory Standards Institute, Wayne, PA, pp. 60–68.

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.

de Neeling, A.J., van den Broek, M.J., Spalburg, E.C., van Santen-Verheuvel, M.G., Dam-Deisz, W.D., Boshuizen, H.C., van de Giessen, A.W., van, D.E., Huijsdens, X.W., 2007. High prevalence of methicillin resistant *Staphylococcus aureus* in pigs. Vet. Microbiol. 122, 366–372.

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.

Finlay, J.E., Miller, L.A., Poupard, J.A., 1997. Interpretive criteria for testing susceptibility of staphylococci to mupirocin. Antimicrob. Agents Chemother. 41, 1137–1139.

Garcia-Alvarez, L., Holden, M.T., Lindsay, H., Webb, C.R., Brown, D.F., Curran, M.D., Walpole, E., Brooks, K., Pickard, D.J., Teale, C., Parkhill, J., Bentley, S.D., Edwards, G.F., Girvan, E.K., Kearns, A.M., Pichon, B., Hill, R.L., Larsen, A.R., Skov, R.L., Peacock, S.J., Maskell, D.J., Holmes, M.A., 2011. Meticillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect. Dis. 11, 595–603.

Gomez-Sanz, E., Torres, C., Lozano, C., Fernandez-Perez, R., Aspiroz, C., Ruiz-Larrea, F., Zarazaga, M., 2010. Detection molecular characterization, and clonal diversity of methicillin-resistant *Staphylococcus aureus* CC398 and CC97 in Spanish slaughter pigs of different age groups. Foodborne Pathog. Dis. 7, 1269–1277.

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.

Holden, M.T., Hsu, L.Y., Kurt, K., Weinert, L.A., Mather, A.E., Harris, S.R., Strommenger, B., Layer, F., Witte, W., de, L.H., Skov, R., Westh, H., Zemlickova, H., Coombs, G., Kearns, A.M., Hill, R.L., Edgeworth, J., Gould, I., Gant, V., Cooke, J., Edwards, G.F., McAdam, P.R., Templeton, K.E., McCann, A., Zhou, Z., Castillo-Ramirez, S., Feil, E.J., Hudson, L.O., Enright, M.C., Balloux, F., Aanensen, D.M., Spratt, B.G., Fitzgerald, J.R., Parkhill, J., Achtman, M., Bentley, S.D., Nubel, U., 2013. A genomic portrait of the emergence, evolution, and global spread of a methicillin-resistant *Staphylococcus aureus* pandemic. Genome Res. 23, 653–664.

Khanna, T., Friendship, R., Dewey, C., Weese, J.S., 2008. Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers. Vet. Microbiol. 128, 298–303.

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.

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.

Lina, G., Piemont, Y., Godail-Gamot, F., Bes, M., Peter, M.O., Gauduchon, V., Vandenesch, F., Etienne, J., 1999. Involvement of Panton-Valentine leukocidin—producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin. Infect. Dis. 29, 1128–1132.

MacGowan, A.P., Wise, R., 2001. Establishing MIC breakpoints and the interpretation of in vitro susceptibility tests. J. Antimicrob. Chemother. 48 (Suppl. 1), 17–28.

Mellmann, A., Weniger, T., Berssenbrugge, C., Rothganger, J., Sammeth, M., Stoye, J., Harmsen, D., 2007. Based Upon Repeat Pattern (BURP): an algorithm to characterize the long-term evolution of *Staphylococcus aureus* populations based on *spa* polymorphisms. BMC Microbiol. 7, 98.

Morgan, M., 2008. Methicillin-resistant *Staphylococcus aureus* and animals: zoonosis or humanosis? J. Antimicrob. Chemother. 62, 1181–1187.

Neela, V., Mohd, Z.A., Mariana, N.S., van, B.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.

Price, L.B., Stegger, M., Hasman, H., Aziz, M., Larsen, J., Andersen, P.S., Pearson, T., Waters, A.E., Foster, J.T., Schupp, J., Gillece, J., Driebe, E., Liu, C.M., Springer, B., Zdovc, I., Battisti, A., Franco, A., Zmudzki, J., Schwarz, S., Butaye, P., Jouy, E., Pomba, C., Porrero, M.C., Ruimy, R., Smith, T.C., Robinson, D.A., Weese, J.S., Arriola, C.S., Yu, F., Laurent, F., Keim, P., Skov, R., Aarestrup, F.M., 2012. *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. mBio 3, e00305–e00311.

Schijffelen, M.J., Boel, C.H., van Strijp, J.A., Fluit, A.C., 2010. Whole genome analysis of a livestock-associated methicillin-resistant *Staphylococcus aureus* ST398 isolate from a case of human endocarditis. BMC Genomics 11, 376.

Sergio, D.M., Koh, T.H., Hsu, L.Y., Ogden, B.E., Goh, A.L., Chow, P.K., 2007. Investigation of meticillin-resistant *Staphylococcus aureus* in pigs used for research. J. Med. Microbiol. 56, 1107–1109.

Smith, T.C., Male, M.J., Harper, A.L., Kroeger, J.S., Tinkler, G.P., Moritz, E.D., Capuano, A.W., Herwaldt, L.A., Diekema, D.J., 2009. Methicillin-resistant *Staphylococcus aureus* (MRSA) strain ST398 is present in midwestern U.S. swine and swine workers. PLoS ONE 4, e4258.

Tenhagen, B.A., Fetsch, A., Stuhrenberg, B., Schleuter, G., Guerra, B., Hammerl, J.A., Hertwig, S., Kowall, J., Kampe, U., Schroeter, A., Braunig, J., Kasbohrer, A., Appel, B., 2009. Prevalence of MRSA types in slaughter pigs in different German abattoirs. Vet. Rec. 165, 589–593.

Uhlemann, A.C., Porcella, S.F., Trivedi, S., Sullivan, S.B., Hafer, C., Kennedy, A.D., Barbian, K.D., McCarthy, A.J., Street, C., Hirschberg, D.L., Lipkin, W.I., Lindsay, J.A., DeLeo, F.R., Lowy, F.D., 2012. Identification of a highly transmissible animal-independent *Staphylococcus aureus* ST398 clone with distinct genomic and cell adhesion properties. mBio 3, e00012–e00027.

Van Cleef, B.A., Monnet, D.L., Voss, A., Krziwanek, K., Allerberger, F., Struelens, M., Zemlickova, H., Skov, R.L., Vuopio-Varkila, J., Cuny, C., Friedrich, A.W., Spiliopoulou, I., Paszti, J., Hardardottir, H., Rossney, A., Pan, A., Pantosti, A., Borg, M., Grundmann, H., Mueller-Premru, M., Olsson-Liljequist, B., Widmer, A., Harbarth, S., Schweiger, A., Unal, S., Kluytmans, J.A., 2011. Livestock-associated methicillin-resistant *Staphylococcus aureus* in humans, Europe. Emerg. Infect. Dis. 17, 502–505.

Vanderhaeghen, W., Hermans, K., Haesebrouck, F., Butaye, P., 2010. Methicillin-resistant *Staphylococcus aureus* (MRSA) in food production animals. Epidemiol. Infect. 138, 606–625.

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, D.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.

Wang, X., Meng, J., Zhou, T., Zhang, Y., Yang, B., Xi, M., Sheng, J., Zhi, S., Xia, X., 2012. Antimicrobial susceptibility testing and genotypic characterization of *Staphylococcus aureus* from food and food animals. Foodborne Pathog. Dis. 9, 95–101.

Wendlandt, S., Lozano, C., Kadlec, K., Gomez-Sanz, E., Zarazaga, M., Torres, C., Schwarz, S., 2013. 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.

Witte, W., Strommenger, B., Stanek, C., Cuny, C., 2007. Methicillin-resistant *Staphylococcus aureus* ST398 in humans and animals, Central Europe. Emerg. Infect. Dis. 13, 255–258.

Wu, D., Wang, Q., Yang, Y., Geng, W., Wang, Q., Yu, S., Yao, K., Yuan, L., Shen, X., 2010. Epidemiology and molecular characteristics of community-associated methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* from skin/soft tissue infections in a children's hospital in Beijing, China. Diagn. Microbiol. Infect. Dis. 67, 1–8.

Xiao, Y.H., Giske, C.G., Wei, Z.Q., Shen, P., Heddini, A., Li, L.J., 2011. Epidemiology and characteristics of antimicrobial resistance in China. Drug Resist. Updat. 14, 236–250.

Zhang, K., McClure, J.A., Elsayed, S., Louie, T., Conly, J.M., 2005. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome *mec* types I to V in methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. 43, 5026–5033.

Zhang, W., Hao, Z., Wang, Y., Cao, X., Logue, C.M., Wang, B., Yang, J., Shen, J., Wu, C., 2011. Molecular characterization of methicillin-resistant *Staphylococcus aureus* strains from pet animals and veterinary staff in China. Vet. J. 190, e125–e129.

Zhao, C., Liu, Y., Zhao, M., Liu, Y., Yu, Y., Chen, H., Sun, Q., Chen, H., Jiang, W., Liu, Y., Han, S., Xu, Y., Chen, M., Cao, B., Wang, H., 2012. Characterization of community acquired *Staphylococcus aureus* associated with skin and soft tissue infection in Beijing: high prevalence of PVL$^+$ ST398. PLoS ONE 7, e38577.

Zhu, Y.G., Johnson, T.A., Su, J.Q., Qiao, M., Guo, G.X., Stedtfeld, R.D., Hashsham, S.A., Tiedje, J.M., 2013. Diverse and abundant antibiotic resistance genes in Chinese swine farms. Proc. Natl. Acad. Sci. U.S.A. 110, 3435–3440.