SHORT COMMUNICATION

Identical genotypes of community-associated MRSA (ST59) and livestock-associated MRSA (ST9) in humans and pigs in rural China

Z. Bi1,* | C. Sun2,* | S. Börjesson3 | B. Chen1 | X. Ji2 | B. Berglund4 | M. Wang1 | M. Nilsson4 | H. Yin4 | Q. Sun5,6 | A. Hult7 | Y. Wang2 | C. Wu2 | Z. Bi1 | L. E. Nilsson4

1Shandong Provincial Key Laboratory of Infectious Disease Control and Prevention, Shandong Center for Disease Control and Prevention, Jinan, China
2Beijing Key Laboratory of Detection Technology for Animal-Derived Food Safety, College of Veterinary Medicine, China Agricultural University, Beijing, China
3Department of Animal Health and Antimicrobial Strategies, National Veterinary Institute (SVA), Uppsala, Sweden
4Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden
5School of Health Care Management, Shandong University, Shandong, China
6Key Laboratory of Health Economics and Policy Research, NHFPC (Shandong University), Shandong, China
7Public Health Agency of Sweden, Solna, Sweden

Correspondence
Zhenqiang Bi, Shandong Provincial Key Laboratory of Infectious Disease Control and Prevention, Shandong Center for Disease Control and Prevention, Jinan, China. Email: bzq63@163.com

Congming Wu, Beijing Key Laboratory of Detection Technology for Animal-Derived Food Safety, College of Veterinary Medicine, China Agricultural University, Beijing, China. Email: wucm@cau.edu.cn

Summary
This study investigated the prevalence of MRSA in samples taken in households, with and without backyard pigs in villages in a rural area of Shandong Province, China. Community-associated MRSA and livestock-associated MRSA, belonging to ST59 and ST9, respectively, were identified in both humans and pigs. The genotypic and phenotypic comparison of isolates indicates that bidirectional transmission of MRSA has occurred between humans and pigs in the villages.

KEYWORDS
community-associated, livestock-associated, methicillin-resistant Staphylococcus aureus, transmission

1 | INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) is a major multidrug-resistant opportunistic pathogen that is responsible for nosocomial infections worldwide. Initially, MRSA was associated with hospital or healthcare contact. However, community-associated MRSA (CA-MRSA) soon emerged worldwide in persons without healthcare contact (Chuang & Huang, 2013; Otter & French, 2010; Vandenesch et al., 2003). During the last decade, livestock-associated MRSA (LA-MRSA) has also emerged. LA-MRSA occurs primarily in pigs and foremost as asymptomatic carriage. CA-MRSA in Asia appears to be characterized mainly by genetic diversity (Chuang & Huang, 2013), but in China, ST59 is the major epidemic clonal lineage of MRSA (Chuang & Huang, 2013). The ST9 lineage
predominates in pigs in most Asian countries. However, the dominant European lineage, CC398, also occurs (Chuang & Huang, 2015; van Cleef et al., 2011), LA-MRSA has been shown to be transmissible from pigs to humans, especially to persons in close contact with animals, and can cause infections in humans (van Duijkeren et al., 2016; Graveland, Duim, van Duijkeren, Heederik, & Wagenaar, 2011). Although the transfer ability of LA-MRSA among humans is lower than that of other strains of MRSA, CC398 is increasing in clinical settings (Hetem, Bootsma, Troelstra, & Bonten, 2013). Human and animal health care are interlinked, and a One Health approach should be applied to understand the dissemination of MRSA between the human and the animal sectors (Cars et al., 2016).

The aim of this study was to investigate the prevalence and epidemiological characteristics of MRSA in villages in a rural area of Shandong Province, China, where humans and backyard pigs were sampled (Sun et al., 2017). Genotypic characterizations of isolates were performed to evaluate relatedness between isolates from humans and pigs.

2 | MATERIALS AND METHODS

2.1 | Samples and population

Sampling from humans and pigs was conducted in 12 villages during July 2015. The aim was to include every household that had a backyard farm with pigs, adding households without backyard farms to reach a total of 65 households per village. In each household, one human nasal sample and skin samples from behind the ear of 1–3 pigs per pen (where relevant) were collected using the ESwab™ collection kit (Copan, Brescia, Italy) according to the manufacturer's instructions.

2.2 | MRSA isolation and identification

From ESwab™ tubes, 0.2 ml of transport liquid was transferred to 1.3 ml 7.5% sodium chloride broth (LandBridge, Beijing, China) and incubated overnight at 35°C. Samples from the overnight broth were cultured on CHROMagar™ MRSA (CHROMagar Company, Paris, France). One colony on each plate suspected of being MRSA was selected and subcultured on sheep blood agar at 35°C overnight and confirmed as *S. aureus* by matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF-MS). For human isolates, Shimadzu Systems (Shimadzu Biotech Corp., Kyoto, Japan) was used, whereas Bruker Daltonik GmbH (Bruker, Bremen, Germany) was used for pig isolates. MRSA isolates were confirmed with PCR by detecting mecA and mecC (Stegger et al., 2012).

2.3 | Detection of PVL and Molecular typing

DNA was extracted from MRSA isolates using TIANamp Bacteria DNAKit (Tiangen, China) and evaluated for occurrence of Panton-Valentine leucocidin (PVL) by PCR (Stegger et al., 2012). All isolates were characterized by multilocus sequence typing (MLST), spa-, SCCmec- and *dru*-typing (Enright, Day, Davies, Peacock, & Spratt, 2000; Goering, Morrison, Al-Douri, Edwards, & Gemmell, 2008; Harmsen et al., 2003; Zhang, McClure, Elsayed, Louie, & Conly, 2005).

2.4 | Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) of isolates was performed using agar dilution with 2-fold antibiotic dilutions in Muller–Hinton agar (Oxoid, Basingstoke, UK) for cefoxitin (FOX), vancomycin (VAN), linezolid (LZD), florfenicol (FLO), gentamicin (GEN), streptomycin (STR), ciprofloxacin (CIP), rifampicin (RIF), tigecycline (TGC), clindamycin (CLI), erythromycin (ERY), tiamulin (TIA), trimethoprim/sulphamethoxazole (SXT), fusidic acid (FUS) and tetracycline (TET). Susceptibility was determined using epidemiological cut-off values (ECOFFs) by the European Committee of Antimicrobial Susceptibility Testing (EUCAST, 2017). *S. aureus* ATCC29213 was used as the quality control strain.

3 | RESULTS

In total, 404 pig ear swab samples and 753 human nasal samples were collected from 245 and 753 households in the 12 villages, respectively (Figure 1). In total, 20 isolates were identified as MRSA with mecA and all negative for PVL. Human isolates of MRSA were identified from 13 (1.7%) households (from seven different villages); pig isolates of MRSA were identified from seven (2.8%) households (from five different villages). In three households (F012, H022 and K046), both human and pig isolates of MRSA were identified.

Molecular typing results showed that ten isolates belonged to ST9-SCCmecIVb-t899-dt12w (five identified among humans and five identified among pigs), seven isolates belonged to ST59-SCCmecIVa-t437-dt10a (six identified among humans and one identified among pigs) and one human isolate of MRSA belonged to ST59-SCCmecIVa-t3257-dt10a which was a single-locus variation in *spa* type IVa-dt10a (six identified among humans and one identified among pigs) and one human isolate of MRSA belonged to ST59-SCCmecIVa-t3257-dt10a which was a single-locus variation in *spa* type IVa-dt10a (six identified among humans and one identified among pigs) and one human isolate of MRSA belonged to ST59-SCCmecIVa-t3257-dt10a which was a single-locus variation in *spa* type IVa-dt10a (six identified among humans and one identified among pigs). The AST (Table 1) showed that all isolates were resistant to ERY, CLI, STR, FOX and susceptible to LZD, VAN and FUS. The ST9 isolates...
also showed resistance to SXT, FLO, CIP, GEN, TGC, TIA and TET. Furthermore, three ST9 isolates were also resistant to RIF.

4 | DISCUSSION

In this study, the prevalence of MRSA in rural residents was investigated. Human isolates of MRSA were identified in 1.7% of the households, which is similar to earlier reports, 2.2% among interns (Ma et al., 2011) and 3.0% carriage among healthy people on a medical college campus in China (Du et al., 2011), although it is higher than the 0.3% carriage reported among adults from Beijing and Harbin (Yan et al., 2015). Another study showed that no MRSA was identified among military volunteers from Beijing (Qu et al., 2010).

The prevalence of MRSA in backyard pigs was also investigated in this study. About 2.8% of the households with sampled backyard farms had pigs with MRSA, which is lower than the prevalence reported earlier from pigs in China (Fan, Wu, Li, Wang, & Zuo, 2014). However, direct comparisons are problematic to make since other studies from China often report prevalence in pigs from a small number of farms and in-herd prevalence, thus possibly reflecting high proportions of carriage at specific farms. Furthermore, large commercial farms are more well studied and may in general have higher MRSA rates, for example, 11% of pigs at commercial pig farms in Henan Province (Fan et al., 2014). Nevertheless, in one study, 21% of small private farms had pigs with MRSA (Yan et al., 2015), which is higher than that in our study.

MLST results showed that all isolates belonged to either ST9 or ST59 (Table 1). ST9 is the LA-MRSA predominating in China and Asian countries (Chuang & Huang, 2015), whereas ST59 is the most common ST among CA-MRSA and MRSA cases in hospitals in China (Chuang & Huang, 2013). ST9 isolates were equally distributed among the human and pig samples, while ST59 isolates were mainly identified among humans, except for one isolate which was recovered from a pig sample. In three households, humans and pigs shared the same genotypes. The findings of households with genotypically identical MRSA isolates of ST59 and ST9 in both pigs and humans indicate that the transmissions of MRSA between humans and animals likely are bidirectional.

The AST (Table 1) showed that the ST9 isolates were resistant to a higher number of antibiotics compared to the ST59 isolates. Three ST9 isolates were also resistant to RIF. RIF resistance has recently been described in LA-MRSA strains that were recovered from pigs at farms and slaughterhouses (Li et al., 2016).

To our knowledge, this is the first study on the prevalence and epidemiological characteristics of MRSA among rural residents and domesticated pigs in China. The data showed an even distribution of CA-MRSA and LA-MRSA among humans and a predominance of
## Table 1: Molecular type and MIC values of MRSA isolates from humans and pigs

| Isolate   | Village | Household # | Backyard Source | MLST | spa | SCCmec | mecut | dru | FLO (mg/L) | CIP (mg/L) | GEN (mg/L) | TGC (mg/L) | TIA (mg/L) | SXT (mg/L) | TET (mg/L) | RIF (mg/L) | FUS (mg/L) | VAN (mg/L) | LZD (mg/L) | ERY (mg/L) | CLI (mg/L) | STR (mg/L) | FOX (mg/L) |
|-----------|---------|-------------|----------------|------|-----|--------|-------|-----|-----------|-----------|------------|------------|------------|------------|------------|------------|------------|-----------|-----------|-----------|-----------|-----------|-----------|
| ATCC29213 | -       | -           | -              | -    | -   |        |       |     | >8        | >1        | >2         | >0.5       | >2         | >0.5       | >1         | >0.032     | >0.5      | >2        | >4        | >1        | >0.25     | >16       | >4        |
| AF012     | F       | 12          | Yes Human ST9 t899 | IVb  | dt12w| 64     | 16    | 64   | 2        | >64       | 4          | 2          | 0.008      | 0.25       | 2          | 4          | >64        | >64       | >64       | >32       |
| YF012     | F       | 12          | Yes Pig ST9 t899  | IVb  | dt12w| 64     | 16    | >64  | 2        | >64       | 4          | 2          | 0.008      | 0.25       | 2          | 4          | >64        | >64       | >64       | >16       |
| YF037     | F       | 37          | Yes Pig ST9 t899  | IVb  | dt12w| 64     | 16    | 64   | 2        | >64       | 1          | 2          | 0.016      | 0.25       | 1          | 4          | >64        | >64       | >64       | >32       |
| YG029     | G       | 29          | Yes Pig ST9 t899  | IVb  | dt11dg| 8      | 16    | 64   | 2        | >64       | 4          | 2          | 0.016      | 0.25       | 1          | 4          | >64        | >64       | >64       | >32       |
| AG037     | G       | 37          | Yes Human ST9 t899 | IVb  | dt12aj| 64     | 16    | 32   | 2        | >64       | 2          | 2          | 0.016      | 0.25       | 1          | 4          | >64        | >64       | >64       | >64       |
| AH018     | H       | 18          | Yes Human ST9 t899 | IVb  | dt12w| 64     | 16    | 32   | 2        | >64       | 4          | 2          | 0.016      | 0.25       | 1          | 4          | >64        | >64       | >64       | >32       |
| AH022     | H       | 22          | Yes Human ST9 t899 | IVb  | dt12w| 64     | 16    | 32   | 2        | >64       | 2          | 2          | 0.016      | 0.25       | 2          | 4          | >64        | >64       | >64       | >32       |
| YH022     | H       | 22          | Yes Pig ST9 t899  | IVb  | dt12w| 64     | 16    | 32   | 2        | >64       | 2          | 2          | 0.008      | 0.25       | 1          | 4          | >64        | >64       | >64       | >16       |
| AH027     | H       | 27          | Yes Human ST9 t899 | IVb  | dt12w| 64     | 16    | 8    | 2        | >64       | 0.5        | 2          | 0.008      | 0.25       | 1          | 4          | >64        | >64       | >64       | >16       |
| YH030     | H       | 30          | Yes Pig ST9 t899  | IVb  | dt12w| 64     | 16    | >64  | 2        | >64       | 4          | 2          | 0.016      | 0.25       | 1          | 4          | >64        | >64       | >64       | >32       |
| YI040     | I       | 40          | Yes Pig ST9 t899  | IVb  | dt12w| 64     | 16    | 8    | 2        | >64       | 0.12       | 2          | 0.016      | 0.25       | 1          | 4          | >64        | >64       | >64       | >32       |
| AL048     | L       | 48          | Yes Human ST9 t899 | IVb  | dt12w| 64     | 16    | 64   | 2        | >64       | 1          | 2          | 0.008      | 0.25       | 2          | 4          | >64        | >64       | >64       | >16       |
| AC045     | C       | 45          | No Human ST59 t3527 | Iva  | dt10a| 8      | 1     | 1    | 0.25     | 2         | 0.03       | 0.5        | 0.008      | 0.25       | 2          | 2          | >64        | >64       | >64       | >16       |
| AD010     | D       | 10          | Yes Human ST59 t437 | Iva  | dt10a| 8      | 1     | 1    | 0.25     | 2         | 0.03       | 0.5        | 0.008      | 0.25       | 2          | 2          | >64        | >64       | >64       | >32       |
| AD016     | D       | 16          | Yes Human ST59 t437 | Iva  | dt10a| 8      | 1     | 1    | 0.25     | 1         | 0.03       | 0.5        | 0.008      | 0.25       | 2          | 4          | >64        | >64       | >64       | >32       |
| AD057     | D       | 57          | No Human ST59 t437 | Iva  | dt10a| 8      | 1     | 1    | 0.25     | 2         | 0.03       | 0.5        | 0.008      | 0.25       | 2          | 4          | >64        | >64       | >64       | >32       |
| AK017     | K       | 17          | No Human ST59 t437 | Iva  | dt10a| 8      | 1     | 1    | 0.25     | 2         | 0.03       | 0.5        | 0.016      | 0.25       | 2          | 4          | >64        | >64       | >64       | >32       |
| AK046     | K       | 46          | Yes Human ST59 t437 | Iva  | dt10a| 8      | 0.5   | 1    | 0.25     | 1         | 0.03       | 0.5        | 0.016      | 0.25       | 2          | 4          | >64        | >64       | >64       | >32       |
| YK046     | K       | 46          | Yes Pig ST59 t437 | Iva  | dt10a| 8      | 1     | 1    | 0.25     | 1         | 0.03       | 0.5        | 0.016      | 0.25       | 1          | 4          | >64        | >64       | >64       | >16       |
| AL008     | L       | 8           | No Human ST59 t437 | Iva  | dt10a| 8      | 1     | 1    | 0.25     | 2         | 0.03       | 0.5        | 0.016      | 0.25       | 2          | 4          | >64        | >64       | >64       | >32       |

FOX, cefoxitin; VAN, vancomycin; LZD, linezolid; FLO, florfenicol; GEN, gentamicin; STR, streptomycin; CIP, ciprofloxacin; RIF, rifampicin; TGC, tigecycline; CLI, clindamycin; ERY, erythromycin; TIA, tiamulin; SXT, trimethoprim/sulphamethoxazole; FUS, fusidic acid; TET, tetracycline.

*Isolates from the same household are marked in bold and italic.

1Susceptibility was determined using epidemiological cut-off values (ECOFFs) by the European Committee of Antimicrobial Susceptibility Testing (EUCAST, 2017). Epidemiological cut-off values are displayed below each antibiotic. Grey cells denote resistance.
LA-MRSA among pigs. In one household, CA-MRSA isolates with shared genotypes could be isolated from both human and pigs, implying that transmission of CA-MRSA from human to pig can take place.

ACKNOWLEDGEMENTS
The authors are grateful for the efforts of the IMPACT consortium: www.folkhalsomyndigheten.se/impact/. This study was funded by National Natural Science Foundation of China [grant number 81361138021, 31472237]; Swedish Research Council [grant number D0879801]; Shandong Provincial Natural Science Foundation [grant number BS2014SW020].

CONFLICT OF INTEREST
There was no conflict of interest.

ETHICS APPROVAL
Ethics approval was obtained from the First Affiliated Hospital, College of Medicine, Zhejiang University, China, reference number 2015#185 and 2015#283.

REFERENCES
Cars, O., Xiao, Y., Stålsby Lundborg, C., Nilsson, L. E., Shen, J., Sun, Q., ... Tomson, G. (2016). Building bridges to operationalise one health – A Sino- Swedish collaboration to tackle antibiotic resistance. One Health, 2, 139–143. https://doi.org/10.1016/j.1oneht.2016.09.002
Chuang, Y. Y., & Huang, Y. C. (2013). Molecular epidemiology of community-associated meticillin-resistant Staphylococcus aureus in Asia. The Lancet Infectious Diseases, 13, 698–708. https://doi.org/10.1016/S1473-3099(13)70136-1
Chuang, Y. Y., & Huang, Y. C. (2015). Livestock-associated meticillin-resistant Staphylococcus aureus in Asia: An emerging issue? International Journal of Antimicrobial Agents, 45, 334–340. https://doi.org/10.1016/j.ijantimicag.2014.12.007
van Cleef, B. A., Monnet, D. L., Voss, A., Krziwanek, K., Allerberger, F., Struelens, M., ... Klyutmans, J. A. (2011). Livestock-associated meticillin-resistant Staphylococcus aureus in humans, Europe. Emerging Infectious Diseases, 17, 502–505. https://doi.org/10.3201/eid1703.101036
Du, J., Chen, C., Ding, B., Tu, J., Qin, Z., Parsons, C., ... Yu, F. (2011). Molecular characterization and antimicrobial susceptibility of nasal Staphylococcus aureus isolates from a Chinese medical college campus. PLoS ONE, 6, e27328. https://doi.org/10.1371/journal.pone.0027328
van Duijkeren, E., Hengeveld, P., Zomer, T. P., Landman, F., Bosch, T., Haenens, A., & van de Giessen, A. (2011). Livestock-associated methicillin-resistant Staphylococcus aureus from pigs in Henan Province. Chinese Veterinary Science, 44, 1123–1230.
Goering, R. V., Morrison, D., Al-Doori, Z., Edwards, G. F., & Gemmell, C. G. (2008). Usefulness of mec-associated direct repeat unit (dru) typing in the epidemiological analysis of highly clonal methicillin-resistant Staphylococcus aureus in Scotland. Clinical Microbiology & Infection, 14, 964–969. https://doi.org/10.1111/j.1469-0691.2008.02073.x
Graveland, H., Duim, B., van Duijkeren, E., Heederik, D., & Wagenaar, J. A. (2011). Livestock-associated meticillin-resistant Staphylococcus aureus in animals and humans. International Journal of Medical Microbiology, 301, 630–634. https://doi.org/10.1016/j.ijmm.2011.09.004
Harmsen, D., Claus, H., Witte, W., Rothganger, J., Claus, H., Turnwald, D., & Vogel, U. (2003). Typing of meticillin-resistant Staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management. Journal of Clinical Microbiology, 41, 5442–5448. https://doi.org/10.1128/JCM.41.12.5442-5448.2003
Hetem, D. J., Bootsma, M. C., Troelstra, A., & Bonten, M. J. (2013). Transmissibility of livestock-associated meticillin-resistant Staphylococcus aureus. Emerging Infectious Diseases, 19, 1797–1802.
Li, J., Fessler, A. T., Jiang, N., Fan, R., Wang, Y., Wu, C., ... Schwarz, S. (2016). Molecular basis of rifampicin resistance in multiresistant porcine livestock-associated MRSA. Journal of Antimicrobial Chemotherapy, 71, 3313–3315. https://doi.org/10.1093/jac/dkw294
Ma, X. X., Sun, D. D., Wang, S., Wang, M. L., Li, M., Shang, H., ... Luo, E. J. (2011). Nasal carriage of meticillin-resistant Staphylococcus aureus among pre-clinical medical students: Epidemiologic and molecular characteristics of meticillin-resistant S. aureus clones. Diagnose Microbiology and Infectious Disease, 70, 22–30. https://doi.org/10.1016/j.diagmicrobio.2010.12.004
Otter, J. A., & French, G. L. (2010). Molecular epidemiology of community-associated meticillin-resistant Staphylococcus aureus in Europe. The Lancet Infectious Diseases, 10, 227–239. https://doi.org/10.1016/S1473-3099(10)70053-0
Qu, F., Cui, E., Guo, T., Li, H., Chen, S., Liu, L., ... Tang, Y. W. (2010). Nasal colonization of and clonal transmission of meticillin-susceptible Staphylococcus aureus among Chinese military volunteers. Journal of Clinical Microbiology, 48, 64–69. https://doi.org/10.1128/JCM.01572-09
Stegger, M., Andersen, P. S., Kearns, A., Pichon, B., Holmes, M. A., Edwards, G., ... Larsen, A. R. (2012). Rapid detection, differentiation and typing of meticillin-resistant Staphylococcus aureus harbouring either mecA or the new mecA homologue mecA(LGA251). Clinical Microbiology & Infection, 18, 395–400. https://doi.org/10.1111/j.1469-0691.2011.03715.x
Sun, Q., Wang, Y., Hult, A., Xiao, Y., Nilsson, L. E., Li, X., ... Stålsby Lundborg, C. (2017). Study protocol for One Health data collections, analyses and intervention of the Sino-Swedish Integrated Multi-sectoral Partnership for Antibiotic Resistance Containment (IMPACT). BMJ Open, 7, (Accepted).
Vandenesch, F., Naimi, T., Enright, M. C., Lina, G., Nimmer, G. R., Fefferman, H., ... Etienne, J. (2003). Community-acquired meticillin-resistant Staphylococcus aureus carrying Panton-Valentine leukocidin genes: Worldwide emergence. Emerging Infectious Diseases, 9, 978–984. https://doi.org/10.3201/eid0908.030089
Yan, X., Song, Y., Yu, X., Tao, X., Yan, J., Luo, F., ... Grundmann, H. (2015). Factors associated with Staphylococcus aureus nasal carriage among healthy people in Northern China. Clinical Microbiology & Infection, 21, 157–162. https://doi.org/10.1016/j.cmi.2014.08.023
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 meticillin-resistant Staphylococcus aureus. Journal of Clinical Microbiology, 43, 5026–5033. https://doi.org/10.1128/JCM.43.10.5026-5033.2005

How to cite this article: Bi Z, Sun C, Börjesson S, et al. Identical genotypes of community-associated MRSA (ST59) and livestock-associated MRSA (ST9) in humans and pigs in rural China. Zoonoses Public Health. 2018;65:367–371. https://doi.org/10.1111/zph.12443