

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

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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

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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 DNABit (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,

Impacts

- Prevalence and epidemiological characteristics of MRSA were studied in rural China.
- CA-MRSA ST59 and LA-MRSA ST9 clones were identified in both humans and pigs.
- Bidirectional transmission of MRSA may have occurred between humans and pigs.

2000; Goering, Morrison, Al-Doori, 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 t437 (Table 1). The other two isolates belonged to ST9-SCCmecIVb-t899-dt12aj (recovered from human sample) and ST9-SCCmecIVb-t899-dt11dg (recovered from pig sample). In the three households, humans and pigs shared the same genotypes, two households with ST9-t899-SCCmecIVb-dt12w and one with ST59-t437-SCCmecIVa-dt10a (Table 1).

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

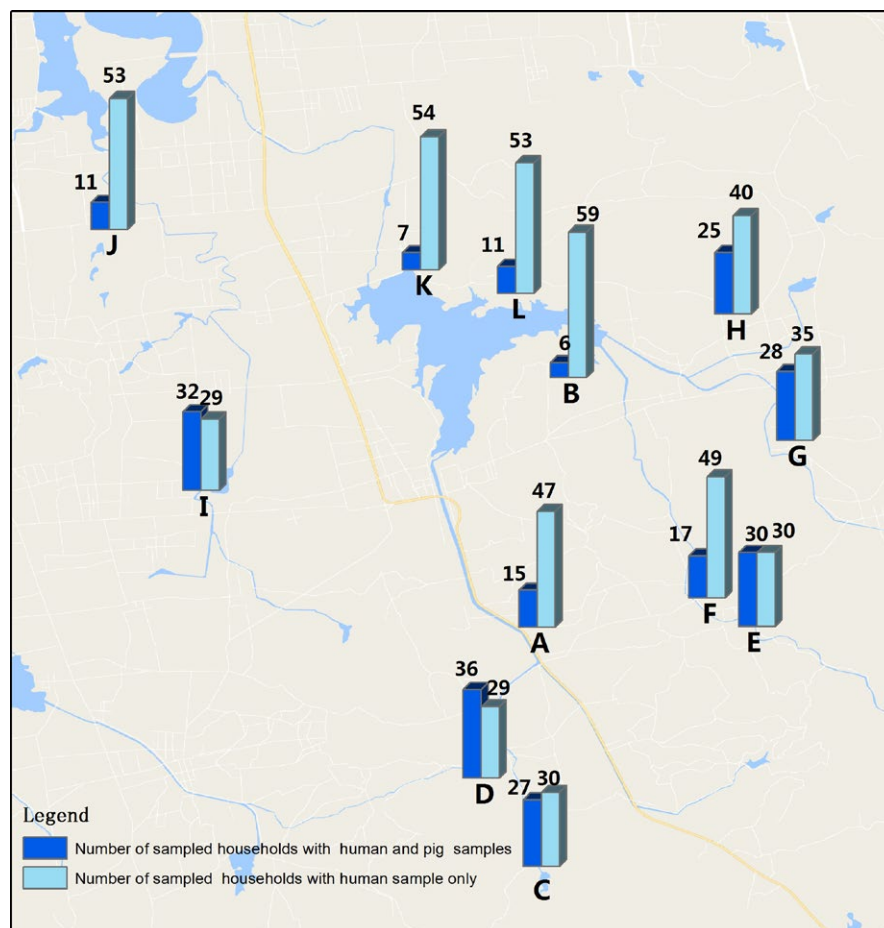


FIGURE 1 Location of 12 villages and number of sampled households of each village. [Colour figure can be viewed at wileyonlinelibrary.com]

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 ^a	Village	Household #	Backyard farm	MIC (mg/L) of ^b																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
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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.

^aIsolates from the same household are marked in bold and italic.

^bSusceptibility 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.

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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.

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