**Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from swine and workers in China**

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**Objectives:** The objectives of this study were to determine the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) colonization in livestock and related workers in four Chinese provinces and the characteristics of these isolates.

**Methods:** Nasal swabs were collected from animals and farm workers in four Chinese provinces. MRSA isolates were recovered and characterized by PFGE, Panton–Valentine leucocidin PCR, staphylococcal chromosomal cassette (SCC) mec typing, spa typing, multilocus sequence typing, antimicrobial susceptibility testing and testing for inducible clindamycin resistance.

**Results:** A total of 60 MRSA isolates were recovered from swine and swine workers. Two predominant multidrug resistance profiles were identified: ciprofloxacin/clindamycin/erythromycin/cefoxitin/gentamicin/tetracycline/chloramphenicol and ciprofloxacin/clindamycin/erythromycin/cefoxitin/gentamicin/tetracycline. All isolates were determined to be spa type t899, contained the group III SCCmec element and were Panton–Valentine leucocidin negative. Multilocus sequence type ST9 (n=46) was identified as the dominant sequence type. One dominant PFGE cluster and a dominant strain type were identified.

**Conclusions:** MRSA from Chinese pigs and farm workers (ST9) differed from the European pig-associated clone (ST398) with regard to clonal type, SCCmec content and resistance profile.

Keywords: antimicrobial susceptibility testing, multilocus sequence typing, pulsed field gel electrophoresis

**Introduction**

While long recognized as a nosocomial infection, the epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) has changed in recent years with the emergence of community-acquired MRSA (CA-MRSA). Recently, contact with livestock, especially swine, has been identified as a risk factor for MRSA carriage in the Netherlands and Canada. Sequence type (ST) 398 has predominated in most reports of MRSA in swine and swine farmers,¹ and concern has been raised about the potential role of swine as a source of CA-MRSA infections through contact with swine, pork products or the farm environment.¹

Although China has a large swine production industry and the largest human population in the world, no study has been performed on the characteristics of swine MRSA isolates. The objective of this study was to determine the prevalence of MRSA colonization in livestock and related workers in four Chinese provinces.

**Materials and methods**

**Sample collection**

From March to July 2008, nasal swabs (Amies agar transport swabs, Copan) were collected from animals and animal workers in ShanXi (15 swine farms and 15 cattle farms), HeBei (16 swine farms and 15 cattle farms), SiChuan (one swine slaughterhouse) and HuBei provinces (two swine slaughterhouses). Five to 10 animal nasal swabs were collected from each farm. Written permission was obtained from all sampled workers.

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**MRSA isolate recovery**

All swabs were aerobically incubated in a broth containing 1% tryptone, 7.5% sodium chloride, 1% mannitol and 0.25% yeast extract at 35 ± 1°C. After 22–24 h incubation, a loopful of the culture was inoculated onto selective MRSA agar plates (BBL CHROMagar MRSA, BD, China) and incubated at 35 ± 1°C for 24–48 h. Purple colonies on the selective plates were screened for coagulase activity. All presumptive MRSA isolates were further investigated by the API Staph ID test (BioMérieux, Beijing, China) and PCR screening for the carriage of *nuc* and *mecA*. All MRSA isolates were stored at −80°C.

**Antimicrobial susceptibility testing**

Antimicrobial susceptibility was determined via broth microdilution, and interpreted according to the CLSI interpretive standards. The MICs of 13 antimicrobials were measured, including cefoxitin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, linezolid, nitrofurantoin, teicoplanin, tetracycline, tigecycline, trimethoprim/sulfamethoxazole and vancomycin. *S. aureus* ATCC 29213 was included as a quality control organism.

**Molecular characterization of the MRSA isolates**

Multilocus sequence typing (MLST) analysis was conducted by sequencing fragments of seven housekeeping genes (*arcC, aroE, gmk, pta, ipp and symL*) and STs were assigned by comparison with the *S. aureus* MLST database (http://www.mlst.net/). The staphylococcal chromosomal cassette (SCC) *mec* types were determined as described by Zhang et al. The polymorphic X-region of the protein A gene (*spa*) was amplified and sequenced. The *spa* types were assigned using an online *spa* database (http://www.spaserver.ridom.de/). The gene encoding Panton–Valentine leucocidin (PVL) was also amplified as previously described. All MRSA isolates were also analysed by PFGE using digestion by Smal (New England Biolabs, Beijing LTD) as previously described. Interpretation of the PFGE patterns was aided by the use of the BioNumerics program (version 4.0; Applied-Maths, Kortrijk, Belgium) using Dice coefficients and the unweighted-pair group method with arithmetic means. Strain types and clusters were defined by DNA banding pattern similarities of 95% and 90%, respectively.

**Results**

**Epidemiological data of samples**

In total, 509 swine nasal swabs, 276 cattle nasal swabs, 167 human nasal swabs (including 107 slaughterhouse workers, 47 cattle workers and 13 swine workers) were collected and screened. Sixty MRSA isolates were recovered from swine (*n* = 58) and swine farm workers (*n* = 2). All 60 isolates harboured *nuc* and *mecA*. No MRSA isolates were recovered from cattle, cattle workers and slaughterhouse workers.

Among 31 swine farms, MRSA isolates were recovered from 13 (41.9%) farms, including four farms (4/15, 26.7%) in ShanXi province and nine farms (9/16, 56.3%) in HeBei province. MRSA isolates were also recovered from one out of two slaughterhouses in SiChuan province and one slaughterhouse in Hubei province (Table 1).

**Antimicrobial susceptibility**

All 60 MRSA isolates were resistant to cefoxitin, ciprofloxacin, clindamycin and tetracycline. A total of four antimicrobial resistance profiles were identified. Two predominant multidrug resistance profiles were identified: ciprofloxacin/clindamycin/erythromycin/cephalosporin/tetracycline/chloramphenicol resistance (*n* = 42) and ciprofloxacin/clindamycin/erythromycin/cefotaxime/gentamicin/tetracycline resistance (*n* = 16) (Figure 1). All isolates were susceptible to linezolid, nitrofurantoin, teicoplanin, tigecycline, trimethoprim/sulfamethoxazole and vancomycin.

**Molecular characterization of MRSA isolates**

All 60 MRSA isolates shared the identical *spa* type (allelic profile, 07-16-23-02-34, i899), contained SCCmec III and were PVL negative. After MLST analysis, three related STs, ST9 (allelic profile 3-3-1-1-1-1-10, 46 isolates), ST912 (allelic profile 3-73-1-1-1-1-10, 13 isolates) and ST1297 (allelic profile 3-203-1-1-1-1-10, one isolate), were identified. These three MLST types differed from each other by one point mutation in *aroE*. MRSA isolates of ST9 were recovered from all four sampled provinces, while isolates of ST912 were only recovered from three pig farms in HeBei province.

Overall, 10 PFGE strain types (A–J) and four clusters (1–4) were identified among the 60 MRSA isolates (Figure 1). The dominant PFGE strain type C (C1–C4) contained 28 isolates originating from all four sampled provinces. Three PFGE strain types were recovered from one farm in ShanXi (Farm E1) and one farm in HeBei province (Farm S5). Two PFGE strain types were identified from two farms in HeBei and two farms in ShanXi province. Six PFGE strain types were identified in a slaughterhouse of HuBei province. From a farm in ShanXi province, identical PFGE patterns were identified between two MRSA isolates originating from a swine worker (isolate HB2) and a swine nasal swab.

| Table 1. MRSA isolates recovered from swine and workers on farms or in slaughterhouses |
|-----------------------------------------------|------------|----------|----------|----------|----------|
| **HeBei** | **HuBei** | **ShanXi** | **SiChuan** | **Total** |
| No. of swine farms with MRSA | 9 (*n* = 16) | — | 4 (*n* = 15) | — | 13 (*n* = 31) |
| No. of slaughterhouses with MRSA | — | 1 (*n* = 1) | — | 1 (*n* = 2) | 2 (*n* = 3) |
| No. of MRSA isolates recovered from swine | 28 (*n* = 112) | 16 (*n* = 132) | 12 (*n* = 141) | 2 (*n* = 124) | 58 (*n* = 509) |
| No. of MRSA isolates from swine farm workers | — | — | 2 (*n* = 13) | — | 2 (*n* = 13) |
| No. of MRSA isolates from slaughterhouse workers | — | 0 (*n* = 91) | — | 0 (*n* = 16) | 0 (*n* = 107) |

*aIndicates no samples were collected.*
Figure 1. Dendrogram of patterns generated by PFGE of MRSA isolates. Statistical analysis of restriction patterns was performed with BioNumerics software (Applied Maths, St-Martens-Latem, Belgium) using the Dice Similarity coefficient. The tree indicating relative genetic similarity was constructed on the basis of the Unweighted Pair Group Method of Averages (UPGMA), position tolerance of 1%; values of 100% mean that the strains are identical. To be considered part of a cluster, the DNA patterns could not differ from each other by more than 10%. Similarity that differed by <5% was considered to represent subtypes within the main group (e.g. A1, A2 and A3). FOX, cefoxitin; CHL, chloramphenicol; CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; GEN, gentamicin; TET, tetracycline.
Swine MRSA, China

Discussion

MRSA ST398 was first identified in the Netherlands in 2003, and is now recognized as a dominant sequence type among swine in different countries. In contrast, no MRSA ST398 isolates were detected in four Chinese provinces in this study. MRSA ST9, a minor animal MRSA sequence type in other countries, was identified as the dominant sequence type in Chinese pigs. The wide dissemination of a single MRSA sequence type among Chinese pigs might be the result of the nature of pig production in China, with minimal exchange of pigs with other countries but common exchange of sows between farms in different provinces. In other countries, methicillin-susceptible S. aureus ST9 (MSSA ST9) isolates have also been reported as a dominant clone among pig farmers. Why MRSA ST9 became the dominant sequence type among Chinese swine MRSA isolates is not known. To our knowledge, this is the first study documenting MRSA in Chinese swine and swine workers.

Of these 60 swine-related MRSA isolates from four Chinese provinces, two MRSA typing methods, SCCmec and spa typing, showed no discriminating power, indicating that these swine-associated MRSA isolates are much more closely related than ST398 isolates. Among MRSA ST398 isolates, various spa–SCCmec types have been documented. A unique characteristic of ST398 is the presence of a novel methylase that renders it untypeable by Smal PFGE. All swine-related MRSA isolates in our study could be typed by PFGE after SmaI digestion, but even with this relatively discriminatory typing method, the isolates recovered in this study still displayed limited genetic diversity.

In this study, MRSA ST9 was recovered from 2 out of 13 swine workers, indicating the interspecies transmission of this sequence type. An interesting result in our study was that no MRSA isolates were recovered from 107 swine slaughterhouse workers, although MRSA was detected among swine samples from the same slaughterhouse (Table 1). All slaughterhouses in this study were using hot water baths (58–65 °C) to scald carcasses. A recent study showed that the decimal reduction time of this MRSA isolate was found to be 1.46 to 2.82 min. This may partially explain why no MRSA isolates were recovered from slaughterhouse workers in our study.

In our study, 58 out of 60 MRSA isolates were resistant to most of the commonly used antimicrobials, suggesting that the same drug classes are being frequently used in swine husbandry. No previous studies have ever reported the extensive resistance patterns we observed. Since these 60 MRSA isolates were collected from four geographically distant provinces, this resistance profile might be common among swine MRSA isolates throughout China.

An international study showed that contact with cows had no influence on the rate of MRSA colonization in veterinarians. In our study, no MRSA isolates were recovered from cattle and cattle farm workers, including those located in the same area from which swine were found to carry MRSA. Although S. aureus is known to be one of the most common causes of bovine mastitis, penicillin, rather than other β-lactam antimicrobials, is still the most commonly used antibiotic in China, which may explain the low prevalence of MRSA in cattle.

To our knowledge, this is the first survey in China examining the prevalence and characteristics of swine MRSA isolates, and our data showed the spread of swine MRSA clones in China that differed from clones identified in other countries.

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Transparency declarations

None to declare.

References