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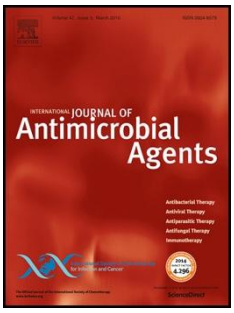

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Emergence of methicillin-resistant *Staphylococcus aureus* ST398 in pigs in China

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Sir,

Staphylococcus aureus, particularly methicillin-resistant *S. aureus* (MRSA), is an important pathogen that causes community and nosocomial infection. The emergence of livestock-associated MRSA (LA-MRSA) is a significant concern for the agriculture industry and public health. The molecular characterization of LA-MRSA showed that isolates differ in diverse geolocations around the world. MRSA sequence type 398 (ST398) isolates have been frequently reported in European and North American countries, whereas most isolates found in Asia belong to the ST9 type [1,2]. We investigated the prevalence and molecular characteristics of pig-associated MRSA in China.

A total of 141 *S. aureus* strains were recovered from the 2997 nasal swab samples collected from pig farms (n = 1815) and hog markets (n = 1182) located in Guangdong, Shanghai, and Shandong. Among them, 104 MRSA (104/141, 73.8%) and 37 methicillin-susceptible *S. aureus* (MSSA) isolates (37/141, 26.2%) were identified by PCR, using the *nuc* gene to detect *S. aureus* and the *nuc* and *mecA* (or *mecC*) for MRSA isolates (Table S1).

All isolates were genotyped by *spa* sequence typing (http://www.ridom.de/spaserver/). MRSA isolates were further analyzed by multilocus sequence typing (MLST) (http://saureus.mlst.net/), and the staphylococcal cassette chromosome *mec* (SCC*mec*) types were determined as described previously (Table S1). Seventeen antibiotics (Table S3) were tested using the agar dilution method according the Clinical and Laboratory Standards Institute (CLSI, 2015) and *S. aureus*

ATCC29213 as a quality control strain. Isolates with a minimum inhibitory concentration (MIC) of ≥ 16 mg/L and ≥ 2 mg/L were considered to be florfenicol and valnemulin resistant, respectively. Resistance genes [*tet(K), tet(L), tet(M), fexA, fexB, cfr*, and *optrA*] were tested by PCR according to previously described primers and procedures (Table S1).

ST9 was the dominant MRSA sequence type in our study (100/104, 96.2%), which was consistent with other reports from Asian countries, including China [2]. Surprisingly, the other four MRSA isolates belonged to ST398. Two of the four ST398-MRSAs were recovered from one pig farm located in Guangdong province, while the other two ST398 were from a pig farm located in Shanghai (Table 1). ST398-MRSA is dominant in Europe and North American, but scarce in China. Only a few studies have reported the presence of ST398 in hospitals, and this is the first report of MRSA ST398 in swine herds in China [3]. We further investigated the environment of the two pig farms, and found that these two farms were located close to a main trunk traffic road. In addition, superior swine breeds and retail meat introduced from Europe or America might be the source of the MRSA ST398 isolates found in this study. For the SCC*mec* types, the four MRSA ST398 isolates were classified as type V, which is the same as those isolates identified in a Shanghai hospital [3]. However, the SCCmec types of all MRSA ST9 isolates were non-typeable, which was similar to a report from Taiwan [4].

The 104 MRSA isolates exhibited nine *spa* types, the most prevalent type being t899 (n = 91, 87.5%). Two *spa* types were identified among the four MRSA ST398

isolates, one was t034 (n = 2), a common and dominant type in Europe and North America [1]; the other was t571 (n = 2), which was previously detected in hospitals in China [3]. Among the 37 MSSA isolates, nine *spa* types were detected. The most common types were t899 (n = 16) and 1775 (n = 9). Two MSSA-t571 isolates recovered from the same farm with MRSA-ST398-t571 in Shanghai were further analyzed by MLST, and were classified as ST398 (Table S2).

The antimicrobial resistance of the MRSA isolates was more serious than that of the MSSA isolates for the majority of the tested antibiotics. Nearly all the *S. aureus* isolates were susceptible to linezolid and rifampicin, and all were susceptible to vancomycin (Table S3). Resistance to tetracycline was mainly mediated by *tet(K)* (n = 29), *tet(L)* (n = 101), and *tet(M)* (n = 12). Florfenicol resistance was mostly encoded by *fexA* (n = 89). Two *cfr*-positive isolates were detected, including one MRSA-ST9-t899 isolate and one MSSA-t4358 isolate collected from a hog market and a pig farm in Guangdong, respectively.

The emergence of MRSA-ST398 isolates in pig farms in China indicated that the epidemiology of MRSA might be changing progressively in swine herds. Pig-associated MRSA are usually multidrug resistant, which might be induced by higher antibiotic usage, both for animal disease treatment and growth promotion [5]. Furthermore, LA-MRSA may be transmitted to humans via the food chain or close contact. As a potential risk to food safety and human health, it is necessary to strengthen our monitoring of MRSA isolates from animals.

Declarations

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Competing Interests: None declared.

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112 **Table 1 Characterization of the pig-associated ST398 MRSA isolates**

Isolates	Region	Time	Origin	*spa*	SCC*mec*	Antimicrobial resistance	Resistance gene
GDC6P096P	Guangdong	2016 May	farm 1	034	V	AMP-OXA-PEN-FOX-TET-FFC-ERY-CLI-TIA-VAL	*mecA-tetK-tetM-fexA*
GDC6P098P	Guangdong	2016 May	farm 1	034	V	AMP-OXA-PEN-FOX-TET-ERY-CLI-TIA-VAL-SXT	*mecA-tetK-tetM*
SHP6P021P	Shanghai	2016 Apr	farm 2	571	V	AMP-OXA-PEN-FOX-TET-FFC-ERY-CLI-TIA-VAL-CIP	*mecA-tetM-fexA*
SHP6P022P	Shanghai	2016 Apr	farm 2	571	V	AMP-OXA-PEN-FOX-TET-FFC-ERY-CLI-TIA-VAL-CIP-SXT	*mecA-tetL-tetM*

113 AMP, ampicillin; OXA, oxacillin; PEN, penicillin; FOX, cefoxitin; TET, tetracycline; FFC, florfenicol; ERY, erythromycin; CLI, clindamycin; TIA, tiamulin; VAL, valnemulin; CIP,

114 ciprofloxacin; SXT, trimethoprim/sulfamethoxazole.

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