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Population deviation of piggery-associated methicillin-resistant *Staphylococcus aureus* based on *mec*-associated direct repeat unit analysis

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ABSTRACT

Piggery-associated methicillin-resistant *Staphylococcus aureus* (MRSA) is a potential zoonotic pathogen. We constructed the population structure and dynamics of staphylococcal chromosome cassette *mec* (SCC*mec*) in MRSA ST9 isolates from different geographical areas of Taiwan. A total of 140 MRSA (135 piggery and 5 human clinical) isolates from three populations located in western Taiwan ($n = 96$ including the 5 clinical isolates), central eastern Taiwan ($n = 22$), and Penghu Island ($n = 22$) were collected and characterized by analysis of the *mec*-associated direct repeat unit (*dru*). Twenty-eight *dru* types (with 24 novel) and 15 *dru*-Clonal Complexes (CCs) were identified. The predominant novel dt12w type (48.6%) was widespread in all populations and may have a superior ability to transmit among populations. The minimum spanning network showed that at least two ancestral *dru* types (dt11a and dt12w) were identified, and the genetics between different populations could be differentiated. Temporal distributions of clone population dynamics estimated through the Bayesian skyline plot indicated a stable population with a long evolutionary history for MRSA ST9 in Taiwan. Findings indicating that some *dru* types are shared between piggery-associated and human-clinical MRSA ST9 suggest the occurrence of cross-species horizontal transmission of SCC*mec*.

1. Introduction

The livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA), first discovered in swine, is becoming a critical zoonotic pathogen worldwide (Gómez-Sanz et al., 2010). MRSA ST9 is frequently reported in livestock animals, meat products, and workers (Feßler et al., 2011; Lo et al., 2012; Neela et al., 2009; Vestergaard et al., 2012). The main mechanism of methicillin resistance in *S. aureus* involves *mecA*, which resides on a mobile genomic element (MGE) called the staphylococcal chromosome cassette *mec* (SCC*mec*) (Deurenberg and Stobberingh, 2008). The SCC*mec* also contains the *ccr* gene complex that is responsible for mobilizing the SCC*mec* element (Deurenberg and Stobberingh, 2008). The *mecA* gene encodes the penicillin-binding protein 2a (PBP2a) with decreased binding affinity for nearly all available β-lactams (Deurenberg and Stobberingh, 2008).

Several molecular typing methods are available for investigating the genetics and clonality of MRSA, such as pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and *spa* typing (Aires de Sousa and de Lencastre, 2004). Another method is SCC*mec* typing, which is based on the determination of the type of *ccr* gene complex and the class of *mec* gene complex (Deurenberg and Stobberingh, 2008). SCC*mec* typing is also commonly used to characterize MRSA by the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC). Several subtypes exist within some SCC*mec* types, based on variations in the "joining regions" around and between the *mec-ccr* gene complexes (Deurenberg and Stobberingh, 2008). However, the SCC*mec* typing results are not always consistent, even when the SCC*mec* structures are the same in different MRSA isolates (Shore et al., 2005; Kim et al., 2007b). A further disadvantage of SCC*mec* typing is the lack of a universal assay for determining the structure of SCC*mec* (Deurenberg and Stobberingh, 2008).

Highly polymorphic variable-number tandem repeat (VNTR) sequences have been applied to study the molecular epidemiology and population genetics of bacterial pathogens (Hidalgo et al., 2010). An advantage of VNTR-based methods is their powerful ability to distinguish and standardize between closely related isolates (Supply et al., 2006). Moreover, the evolution of these polymorphic sites through tandem expansion and contraction also allows researchers to make phylogenetic inferences (Riegler et al., 2012). Recently, a 40-bp VNTR direct repeat unit (*dru*) within the staphylococcal SCC*mec* region adjacent to the region between *mecA* and IS*431* gained wide attention as a marker for distinguishing the

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UK strains of highly clonal epidemic MRSA (EMRSA)-15 and EMRSA-16 MRSA, and acted as an internal "marker" in studies on the horizontal transmission of SCC*mec* (Goering et al., 2008).

Our previous study showed that the prevalence of LA-MRSA ST9 in Taiwan was higher than that in other Asian countries (Lo et al., 2012). LA-MRSA ST9 and human clinical ST9-MRSA isolates share a novel staphylocoagulase (SC) XIc subtype, indicating a recent common ancestry (Wan et al., 2012). However, the diversity of the MGE-SCC*mec* based on the *dru* region in swine and human clinical MRSA ST9 isolates is unknown. Whether the geographical distribution or transportation of swine affects the population diversity of SCC*mec* in different areas is also uncertain. Therefore, the *dru* regions of ST9 MRSA isolates from swine and humans from three geographical areas in Taiwan were analyzed. The phylogeography of ST9 MRSA was then mapped based on *dru* types to examine geographic-genetic patterns, thus allowing us to infer the historical population dynamics of swine SCC*mec* in Taiwan.

2. Material and methods

2.1. Bacterial strains and population definitions

In total, 140 isolates of ST9 MRSA from three geographic areas of Taiwan were collected and analyzed. The selection criteria of the three MRSA populations were based on the swine transportation network and in accordance with the pig-farm location in Taiwan (Fig. 1). The sources of ST9 MRSA isolates were divided into (1) western Taiwan, (2) central eastern Taiwan (Hualian County), and (3) Penghu Island. Western Taiwan isolates were collected from 9 counties in western Taiwan, which housed more than 80% swine population of Taiwan. Due to geographical barrier, pigs of central eastern Taiwan and Penghu Island are traded locally that do not mingle with pigs of western Taiwan. Isolates from western Taiwan ($n = 96$) included our previous 91 ST9 LA-MRSA (Lo et al., 2012) and 5 human clinical isolates (Wan et al., 2012). Central eastern Taiwan isolates ($n = 22$) were collected in 2011 from 30 asymptomatic swine nasal samples. Penghu Island isolates ($n = 22$) were recovered from 32 asymptomatic swine nasal samples in 2010 (unpublished data). All the isolates were determined by coagulase testing and screened by 6 μg/mL oxacillin with 4% NaCl MRSA phenotypic testing, and further confirmed using PCR to detect the *nuc* and *mecA* genes according to our previous published methods (Lo et al., 2012).

2.2. dru typing

Amplification of the *mec*-associated hypervariable region containing variable numbers of 40-bp *dru* was performed by PCR with primers dru-1 (−) and dru-2 (+), as described by Goering et al. (2008). Novel *dru* types were submitted to the *dru* databank (http://www.dru-typing.org).

2.3. dru-clonal complexes clustering

A cluster analysis of *dru* sequences was performed using the multi locus-variable number of tandem repeat model (ML-VNTR) with goeBURST algorithm implemented by Phyloviz 1.0 software (http://goeburst.phyloviz.net/) (Francisco et al., 2009) to determine the relationship among *dru* types. To prevent the error in ML-VNTR with goeBURST inference, the JAVA application eBURST algorithm (v.3) was carried out (Feil et al., 2004). Clonal complexes (CCs) were defined as *dru* types linked through single locus variants (SLVs) from another *dru* type of the group. The components of *dru*-CCs from different populations were assessed using the chi-square test for homogeneity of proportions. A P value of <0.05 was considered significant.

2.4. Population genetics analysis and geographical distribution

DnaSP (v.5.0, Librado and Rozas, 2009) was used to calculate haplotype diversity (h) and nucleotide diversity (π) to determine genetic diversity. DnaSP was also used to calculate parameters for the historical demography of populations. Mismatch distribution analysis (Rogers and Harpending, 1992) was used to detect population expansion events, and gene flow (F_{ST}) for genetic differentiation. A P value of <0.05 was considered significant. A low level of haplotype and nucleotide diversity was defined as $h < 0.5$ and $\pi < 0.5\%$ (Grant and Bowen, 1998). The magnitude of genetic differentiation among the groups of *dru* was determined using the scale proposed by Wright (1978), where F_{ST} values of 0–0.05 indicate low genetic differentiation, 0.05–0.15 moderate, 0.15–0.25 high, and above 0.25 very high. The number of gene flow (Nm) was

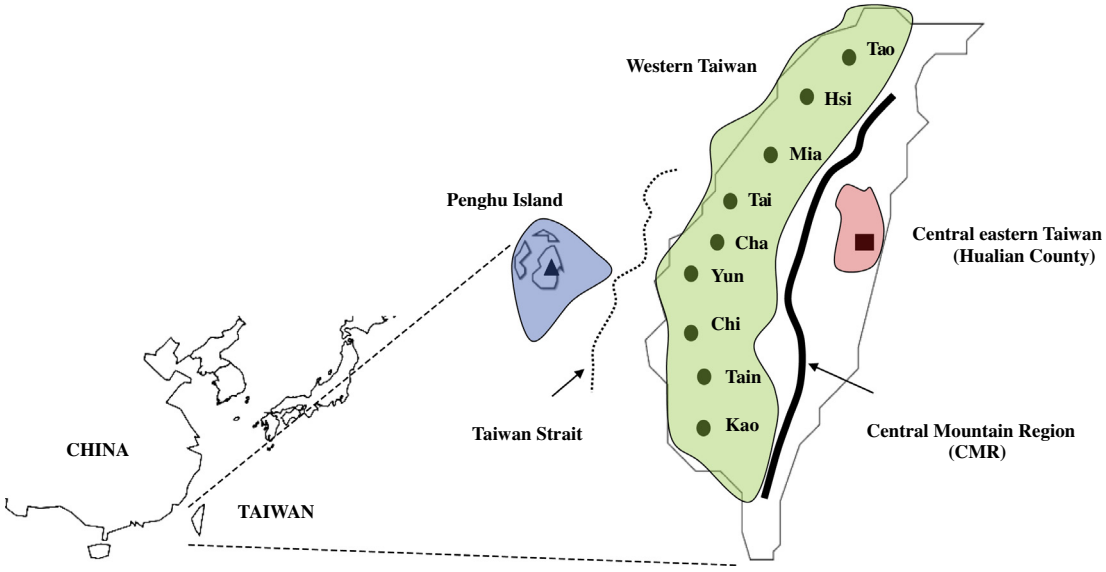

Fig. 1. Sampling locations of 140 MRSA isolates. Western Taiwan contains 9 western counties (black circles): Tao: Taoyuan; Hsi: Hsinchu; Mia: Miaoli; Tai: Taichung; Cha: Changhua; Yun: Yulin; Chi: Chaiyi; Tain: Tainan; Kao: Kaohsiung.