

## Clinical characteristics, virulence factors and molecular typing of methicillin-resistant *Staphylococcus aureus* infections in Shenzhen City, China

L. HU<sup>1,2†</sup>, Y. LI<sup>2†</sup>, Y. LU<sup>2</sup>, J. D. KLENA<sup>3,4</sup>, Y. QIU<sup>2</sup>, Y. LIN<sup>2</sup>, M. JIANG<sup>2</sup>,  
X. SHI<sup>2</sup>, L. CHEN<sup>2</sup>, X. LIU<sup>2</sup>, H. MA<sup>2</sup>, J. CHENG<sup>2</sup>, S. WU<sup>3</sup>,  
B. KAN<sup>5</sup> AND Q. HU<sup>1,2\*</sup>

<sup>1</sup> College of Life Sciences, Shenzhen University, Shenzhen, Guangdong, China

<sup>2</sup> Shenzhen Major Infectious Disease Control Key Laboratory, Shenzhen Center for Disease Control and Prevention, Shenzhen, Guangdong Province, China

<sup>3</sup> United States Centers for Disease Control and Prevention, Beijing, China

<sup>4</sup> Division of Global Health Protection, Center for Global Health, United States Centers for Disease Control and Prevention, Atlanta, GA, USA

<sup>5</sup> Key Laboratory of Surveillance and Early-warning on Infectious Disease, Division of Infectious Disease, Chinese Center for Disease Control and Prevention, Beijing, China

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### SUMMARY

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a serious hospital and community-acquired infection and some strains are associated with greater severity. We investigated the clinical variability and molecular characteristics of MRSA infections in Shenzhen, China through a study at nine sentinel hospitals from January to December 2014. MRSA infections were classified as community-associated (CA-MRSA), healthcare-associated (HA-MRSA), and healthcare-associated community-onset (HACO-MRSA). In total, 812 MRSA isolates were collected and 183 of these were selected for further study. Patients with HA-MRSA infections were generally of greater age compared to other groups. Distinct body site and clinical presentations were evident in infected patients, e.g. CA-MRSA (skin and soft tissue, 53%), HA-MRSA (respiratory tract, 22%; surgical site, 20%; trauma wounds, 20%) and HACO-MRSA (mastitis, 47%). In contrast to HA-MRSA, other categories of strains were significantly more susceptible to gentamicin, sulfamethoxazole/trimethoprim, and tetracycline. No resistance to vancomycin or linezolid was recorded. The predominant clonal lineage within each strain category was CC59-t437-SCCmec IV/V-*agr* I (CA, 51.4%; HA, 28.9%; HACO, 52.9%) which exhibited characteristics of a traditional CA clone together with *agr* I which is more often associated with HA clones. In conclusion, for the three categories of MRSA infections, there were significant differences in clinical characteristics of patients, but the predominant clone in each category shared a similar genetic background which suggests that transmission of MRSA strains has occurred between the community and hospitals in Shenzhen.

**Key words:** CA-MRSA, clinical characteristics, HA-MRSA, HACO-MRSA, methicillin-resistant *Staphylococcus aureus*, molecular typing, virulence factors.

\* Author for correspondence: Dr Q. Hu, Shenzhen Centre for Disease Control and Prevention, Shenzhen 518055, Guangdong Province, China.  
(Email: huqinghua03@163.com)

† These authors contributed equally to this work.

### INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important human and animal pathogen capable

of causing serious outbreaks of infection in both hospital and community patients worldwide [1]. Healthcare-associated MRSA (HA-MRSA) infections are most often associated with surgery, dialysis, hospitalization, use of indwelling catheters or other percutaneous medical devices, and residence in a long-term care facility [2]. In the 1990s, reports appeared of community-acquired infections with MRSA (CA-MRSA) in healthy individuals such as army recruits, sports teams, and prisoners [3] and many of these infections were attributed to genetically novel strains [4]. More recently, a third category of MRSA has been recognized to describe strains that had the genetic characteristics of HA-MRSA but with community onset of infection (HACO-MRSA); the standard epidemiological definitions of infection categories was established by the US Centers for Disease Control and Prevention (US CDC) [5].

Several studies have shown that isolates causing CA- and HA-MRSA infections are distinct in antimicrobial resistance patterns, clinical and molecular characteristics [6], with HA-MRSA strains being typically more resistant to non- $\beta$ -lactam antimicrobials [7]. Moreover, the staphylococcal cassette chromosome *mec* (SCC*mec*) elements conferring methicillin resistance in traditional HA-MRSA strains are most frequently of types I, II, or III whereas SCC*mec* elements IV and V, which are shorter and more easily transferred to other strains, are more often associated with CA-MRSA strains [8]. In addition, the accessory gene regulator (*agr*) type III is reported to be common in CA-MRSA strains, whereas HA-MRSA strains are more likely to be *agr* I or II [6]. However, it is becoming clear that the line between traditional HA-MRSA and CA-MRSA strains based on clinical characterization and susceptibility testing is becoming increasingly blurred [9], with some authors arguing in favour of infection category definitions according to the strain genotype isolated from the patient [10]. These reports imply that it is insufficient to base the definition of MRSA strains only on clinical characteristics.

Against this background we set out to determine the prevalence and clinical characteristics of patients presenting with MRSA infections in both the hospital and community in Shenzhen, a major city in Guangdong Province, located in the southeast of China. Correlates were sought between category of infection according to epidemiological criteria and molecular characteristics and genetic background of MRSA recovered within each infection category.

## METHODS

### Bacterial isolation

Nine sentinel hospitals were included in the project based on their geographical location, population diversity served, and laboratory capacity (Supplementary Fig. S1). These public hospitals serve 2500–6000 outpatients per day and 1000–6500 inpatients per month. In each hospital, clinical specimens were cultured on blood agar and typical staphylococcal colonies were confirmed as *S. aureus* using the Vitek-2 Compact system (bioMérieux, USA). Isolates were screened for resistance to methicillin or oxacillin and MRSA were forwarded to the Shenzhen Center for Disease Control and Prevention (Shenzhen CDC) for further genetic characterization (Supplementary Fig. S2).

### Clinical data

Clinical staff in the infection control department of the sentinel hospitals were responsible for collection of information on all MRSA-infected patients using a standard questionnaire to record demographic data, clinical information, and medical history.

### Case definition

MRSA infections were categorized as HA, CA or HACO according to established criteria [5]. A patient was considered to be colonized with MRSA if the organism was isolated from sputum or throat swabs, but was afebrile, did not have a raised white cell count or a positive chest X-ray.

### Antimicrobial susceptibility

Minimal inhibitory concentrations of gentamicin, ciprofloxacin, sulfamethoxazole-trimethoprim, erythromycin, vancomycin, clindamycin, tetracycline and linezolid were determined in the Vitek-2 Compact system with AST-GP67 susceptibility cards (bioMérieux) according to the Clinical and Laboratory Standards Institute (CLSI) [11]. ATCC29213 (*S. aureus*) and ATCC29212 (*Enterococcus faecalis*) were used as reference controls.

### Virulence genes

Isolates were screened for the *S. aureus*-specific thermonuclease gene (*nuc*), methicillin resistance gene *mecA*, Pantón–Valentine leukocidin (PVL), and 16

enterotoxin genes (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, *seo*, *sep*, *seq*) by polymerase chain reaction (PCR) using conserved primers as described previously and listed in Supplementary Table S1 [12, 13]. DNA was extracted by suspending a single colony in 200 µl of 2% (w/v) Chelex 100 resin slurry (Bio-Rad Laboratories Ltd, Canada) and heating at 95 °C for 10 min. The presence of the *nuc*, *mecA* and PVL genes was determined via a triplex real-time PCR assay, and enterotoxin genes were tested by a single PCR.

### Molecular typing

Isolates were characterized by SCC*mec* type, multilocus sequence type (MLST), staphylococcal protein A gene (*spa*) type, and accessory gene regulator (*agr*) using primers as in previous reports listed in Supplementary Table S1 [14–17]. Two multiplex PCR strategies were used to identify SCC*mec* types I–V; *spa* typing was performed in a single PCR and types were assigned using BioNumerics v. 6.0 software (Applied Maths, Belgium), and each of the four *agr* types was determined by a single PCR. Analysis of allelic profiles and sequence types (STs) were as described on the MLST website (<http://saureus.mlst.net/>), and eBURST v. 3.0 software (<http://eburst.mlst.net/>) was used to identify clonal complexes (CCs). Pulsed-field gel electrophoresis (PFGE) typing was based on *Sma*I restriction profiles as described previously [18] and gel images were analysed using BioNumerics v. 6.0 software. Strain profiles were grouped using Dice coefficients and a 1.5% band-matching criterion (80% relatedness) to define PFGE type. *Xba*I-digested *Salmonella* Braenderup H9812 DNA was used as a size standard.

### Data analysis

Statistical analysis was performed using SPSS v. 16.0 software (SPSS Inc., USA). Demographic, clinical and genetic characteristics of different types of MRSA were compared using  $\chi^2$  or Fisher's exact tests. *P* values <0.05 were considered statistically significant.

### Ethics

The study was approved by the Institutional Review Board at Shenzhen CDC and the US CDC. All

participants gave oral consent for use of their specimens and information for research purposes.

## RESULTS

From January to December 2014, a total of 107 406 clinical specimens were cultured for bacteria at the nine study hospitals, of which 3029 (2.8%) were identified as *S. aureus* and 812 (0.8%) as MRSA. Of these 812 strains, 596 (73.4%) were excluded as colonizing strains and a further 33 (4.1%) could not be classified due to lack of epidemiological information, giving a final study total of 183 (22.5%) MRSA strains considered to have caused clinical infection. The incidence of MRSA from all clinical specimens examined in the study hospitals was 0.2% (183/107406). None of the subjects died in any of the MRSA cases.

By CDC criteria the 183 MRSA infections were categorized as CA-MRSA (70, 38.3%), HA-MRSA (45, 24.6%) and HACO-MRSA (68, 37.2%) (Table 1). Overall, 52.5% of all patients were female but this sex was overrepresented in HACO-MRSA infections (64.7%) compared to CA-MRSA and HA-MRSA cases (*P* = 0.033). Two predominant age groups were evident: 0–14 years (38.8%) and 20–40 years (39.3%) with an older median age in HA-MRSA patients. CA-MRSA strains were most often isolated from the 0–14 years age group (52.9%) whereas HA-MRSA strains were broadly distributed across age groups, and HACO-MRSA strains in the 20–40 years age group (58.8%). Significantly more elderly patients were infected with HA-MRSA strains (*P* < 0.001).

### Clinical characteristics

Table 2 shows that skin and soft tissue infection (SSTI), mastitis, trauma wounds, surgical site, and the respiratory tract were the top five sites of MRSA infections and varied in frequency according to infection category. CA-MRSA occurred most frequently in SSTI (52.9%), while HA-MRSA infections were most associated with the respiratory tract (22.2%), surgical site (20.0%) and trauma wound infections (20.0%). Notably, HACO-MRSA strains were most frequent in mastitis cases (47.1%). Most (89.6%) patients were treated as inpatients, primarily in general surgery (24%), mastitis (12%) and orthopaedic (11.5%) departments. However, it is noteworthy that 21.4% of outpatients were categorized as CA-MRSA infections.

Table 1. Demographic characteristics of CA-MRSA, HA-MRSA and HACO-MRSA of case-patients

Characteristics	Total n (%)	CA-MRSA n (%)	HA-MRSA n (%)	HACO-MRSA n (%)	P value
Isolates	183 (100.0)	70 (38.3)	45 (24.6)	68 (37.2)	
Sex					0.033
Male	87 (47.5)	40 (57.1)	23 (51.1)	24 (35.3)	
Female	96 (52.5)	30 (42.9)	22 (48.9)	44 (64.7)	
Median age (range)	24 (0–86)	23 (0–68)	40 (0–86)	23 (0–55)	
0–14	71 (38.8)	37 (52.9)	10 (22.2)	24 (35.3)	0.003
15–19	5 (2.7)	2 (2.9)	2 (4.4)	1 (1.5)	0.847
20–40	72 (39.3)	20 (28.6)	12 (26.7)	40 (58.8)	<0.001
41–60	23 (12.6)	10 (14.3)	10 (22.2)	3 (4.4)	0.016
>60	12 (6.6)	1 (1.4)	11 (24.4)	0 (0.0)	<0.001

CA-MRSA, Community-associated methicillin-resistant *Staphylococcus aureus*; HA, hospital-associated; HACO, healthcare-associated community-onset.

Table 2. Infectious type and location of patients with CA-MRSA, HA-MRSA and HACO-MRSA

Characteristics	CA-MRSA n (%)	HA-MRSA n (%)	HACO-MRSA n (%)	Total n (%)
Infectious type				
SSTI*	37 (52.9)	3 (6.7)	11 (16.2)	51 (27.9)
Mastitis	2 (2.9)	1 (2.2)	32 (47.1)	35 (19.1)
Trauma wound	11 (15.7)	9 (20.0)	2 (2.9)	22 (12.0)
Surgical site	2 (2.9)	9 (20.0)	8 (11.8)	19 (10.4)
Respiratory tract	1 (1.4)	10 (22.2)	6 (8.8)	17 (9.3)
Blood stream	5 (7.1)	4 (8.9)	4 (5.9)	13 (7.1)
Other	12 (17.1)	9 (20.0)	5 (7.4)	26 (14.2)
Inpatients (department)	55 (78.6)	45 (100.0)	64 (94.1)	164 (89.6)
General surgery	17 (24.3)	10 (22.2)	17 (25.0)	44 (24.0)
Mastitis	0 (0.0)	2 (4.4)	20 (19.4)	22 (12.0)
Orthopaedics	11 (15.7)	8 (17.8)	2 (2.9)	21 (11.5)
Paediatrics	8 (11.4)	1 (2.2)	5 (7.4)	14 (7.7)
Internal medicine	4 (5.7)	6 (13.3)	4 (5.9)	14 (7.7)
Neonate	0 (0.0)	3 (6.7)	10 (14.7)	13 (7.1)
Other†	15 (21.4)	15 (33.3)	6 (8.8)	36 (19.7)
Outpatients	15 (21.4)	0 (0.0)	4 (5.9)	19 (10.4)

CA-MRSA, Community-associated methicillin-resistant *Staphylococcus aureus*; HA, hospital-associated; HACO, healthcare-associated community-onset.

\* SSTI, Skin and soft-tissue infections (epifolliculitis, carbuncle, pustulosis, cellulitis, necrotizing lesions).

† MRSA patients in other departments (neurosurgery, 4.9%; intensive care unit, 3.3%; paediatric intensive care unit, 1.6%).

### Antimicrobial resistance

In the three categories of infections, >80% and 60% of strains were resistant to erythromycin and clindamycin, respectively. Compared to HA-MRSA strains, the other two categories exhibited lower resistance rates for gentamicin ( $P < 0.001$ ), ciprofloxacin ( $P < 0.001$ ), sulfamethoxazole/ trimethoprim ( $P = 0.009$ ) and tetracycline ( $P = 0.005$ ), although over 40% were resistant to tetracycline. All isolates were susceptible

to vancomycin and linezolid (Supplementary Table S2).

### Genetic characterization

All 183 study strains were positive for *nuc* and *mecA* genes, with 51.9% harbouring PVL genes (Table 3); the latter being more frequent in CA- and HACO-MRSA strains. One or more of 9 of the 16

Table 3. Distribution of *nuc*, *mecA*, *PVL* and enterotoxin genes, *SCCmec* and *agr* types in CA-MRSA, HA-MRSA and HACO-MRSA strains

Characteristics	Total n (%)	CA-MRSA n (%)	HA-MRSA n (%)	HACO-MRSA n (%)	P value
<i>Nuc</i>	183 (100.0)	70 (100.0)	45 (100.0)	68 (100.0)	
<i>mecA</i>	183 (100.0)	70 (100.0)	45 (100.0)	68 (100.0)	
<i>PVL</i>	95 (51.9)	48 (68.6)	10 (22.2)	37 (54.4)	<0.001
Any enterotoxin gene	160 (87.4)	58 (82.9)	44 (97.8)	58 (85.3)	0.047
<i>sea</i>	11 (6.0)	1 (1.4)	10 (22.2)	0 (0.0)	<0.001
<i>seb</i>	117 (63.9)	43 (61.4)	25 (55.6)	49 (72.1)	0.168
<i>sec</i>	15 (8.2)	8 (11.4)	3 (6.7)	4 (5.9)	0.485
Other*	17 (9.3)	6 (8.6)	6 (13.3)	5 (7.4)	0.581
Non-typable	23 (12.6)	12 (17.1)	1 (2.2)	10 (14.7)	0.047
<i>SCCmec</i> type					
III	11 (6.0)	0 (0.0)	11 (24.4)	0 (0.0)	<0.001
IV	83 (45.4)	29 (41.4)	20 (44.4)	34 (50.0)	0.594
V	62 (33.9)	26 (37.1)	11 (24.4)	25 (36.8)	0.315
IV/V	145 (79.2)	55 (78.6)	31 (68.9)	59 (86.8)	0.072
Non-typable	27 (14.8)	15 (21.4)	3 (6.7)	9 (13.2)	0.086
<i>agr</i> type					
I	144 (78.7)	54 (77.1)	33 (73.3)	57 (83.8)	0.393
II	10 (5.5)	1 (1.4)	7 (15.6)	2 (2.9)	0.004
III	22 (12.0)	11 (15.7)	3 (6.7)	8 (11.8)	0.354
I/II	154 (84.2)	55 (78.6)	40 (88.9)	59 (86.8)	0.261
Non-typable	7 (3.8)	4 (5.7)	2 (4.4)	1 (1.5)	0.438

CA-MRSA, Community-associated methicillin-resistant *Staphylococcus aureus*; HA, hospital-associated; HACO, healthcare-associated community-onset.

\* Other enterotoxin genes (< 3%).

enterotoxin genes tested were detected in 87.4% of the strains (Table 3) with *seb* (63.9%) being the most dominant in each strain category.

The majority (82.5%) of 183 strains could be allocated to a *SCCmec* type with types IV (45.4%) and V (33.9%) being the most prevalent. No difference in *SCCmec* IV/V presence was evident between the three categories ( $P=0.07$ ) (Table 3). By MLST, 31 STs were identified of which nine were novel to this study. The STs fell into 14 CCs with one singleton (Supplementary Table S3). CC59 (60.7%), predominated in each category followed by CC1 (7.7%) and CC239 (6.6%). Forty-four different *spa* types were found and four were novel; type t437 accounted for 51.9% of all study strains and far exceeded the frequency of the second most common type, t114 (4.9%). Likewise, *agr* type I (78.7%) predominated in all strains within each group; *agr* type IV was not detected, and 3.8% were non-typable (Table 3).

Table 4 shows 36.1% of all strains in six clonal groups according to the USA clonal groupings. USA1000 was represented by 38 (20.8%) strains followed by USA400 (8.7%) with no significant

difference between the categories of infections, but 117 of the total strains were characterized by PFGE profiles distinct from the USA groups.

The predominant clone in CA-, HA- and HACO-MRSA was defined as CC59-t437-*SCCmec* IV/V-*agr* I with a distribution of 51.4% (36/70), 28.9% (13/45) and 52.9% (36/68), respectively. The specific subclone USA1000 within this genetic lineage was identified in 12.9% of CA-, 20% of HA- and 10.3% of HACO-MRSA strains (Table 5).

## DISCUSSION

We performed epidemiological and molecular characterization of 183 clinical MRSA isolates from patients attending nine sentinel hospitals in Shenzhen in 2014 who were categorized as having CA-, HA-, or HACO-MRSA. The top three clones identified were CC59-t437-*SCCmec* IV/V-*agr* I (46.4%), which was dominant across each infection category, CC239-t030/t037-*SCCmec* III-*agr* I (4.4%), and CC1-t114-*SCCmec* IV-*agr* III (3.8%). Older patients were more likely to harbour HA-MRSA strains and the major

Table 4. Distribution of pulsed-field gel electrophoresis pattern types and clonal complexes of CA-MRSA, HA-MRSA and HACO-MRSA strains

PFGE pattern	Clonal complexes (no.)													Total	CA-MRSA	HA-MRSA	HACO-MRSA	P value
	CC1	CC59	CC5	CC9	CC45	CC239	CC30	CC72	Singleton 2580	Others	CA-MRSA	HA-MRSA	HACO-MRSA					
USA400	13	2	0	1	0	0	0	0	0	0	0	0	0	16	5	3	8	0.634
USA600	0	0	0	0	5	0	0	0	0	0	0	0	0	5	2	2	1	0.577
USA700	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0.246
USA800	0	0	3	0	0	0	0	0	0	0	0	0	0	3	1	1	1	1.000
USA1000	0	37	0	0	0	1	0	0	0	0	0	0	0	38	10	10	18	0.213
USA1100	0	0	0	0	0	0	2	0	1	0	0	0	0	3	3	0	0	0.118
Other*	1	72	3	4	0	11	0	0	0	26	117	49	28	117	49	28	40	
Total	14	111	6	5	5	12	2	1	1	26	183	70	45	183	70	45	68	

CA-MRSA, Community-associated methicillin-resistant *Staphylococcus aureus*; HA, hospital-associated; HACO, healthcare-associated community-onset. \* MRSA strains distinct from USA clonal type patterns.

infection sites varied with the category of infection. Overall, our findings suggest that the MRSA strains of different origins shared similar major molecular features but differed by demographic and clinical characteristics of the patient cohorts.

MRSA strains from the three patient groups exhibited high rates of resistance to erythromycin, clindamycin and tetracycline, and HA-MRSA isolates in particular were often also resistant to gentamicin and ciprofloxacin. The antimicrobial resistance profile of the primary clone CC59-t437-SCCmec IV/V-*agr* I strains was consistent with that previously reported for CC59 strains in another study in mainland China [19]. Knowledge of drug resistance rates for MRSA strains is essential for targeting of effective antimicrobial drug therapy and can help to reduce the likelihood of the emergence of other resistant pathogens.

In China, CC59-t437-SCCmec IVa was the most predominant CA-MRSA clone in neonates [20], while CC239-t030/t037-SCCmec III was most frequently recovered from hospital patients during the past year [21]. An earlier study of MRSA strains from nine teaching hospitals in six cities between 2009 and 2012 in Guangzhou, Guangdong Province, China, showed that CC239-t030/t037-SCCmec III was the most predominant MRSA clone [22], but epidemiological classification of patient category was not conducted. Another investigation undertaken 5 years previously over 6 months also identified the latter as the dominant clone in Guangzhou as an HA-MRSA lineage but also reported the isolation of clone CC59-t437-SCCmec IV which is classified as a CA-MRSA lineage [23]. Moreover, a recent comprehensive review of MRSA data from Asian countries demonstrated that in Hong Kong and Taiwan, the major clonal lineage of CA-MRSA strains was ST59-SCCmec IV/V, and the major clone of HA-MRSA strains was ST239-SCCmec III [24]. In contrast with earlier studies in Hong Kong, Taiwan, and mainland China, the CC59-t437-SCCmec IV/V clone appears to have replaced CC239-t030/t037-SCCmec III to become the major HA-MRSA clone in our study. This indicates that CA-MRSA strain lineages may be replacing traditional HA-MRSA strains, as illustrated here by the possible spread of the CC59-t437-SCCmecIV/V clone from the community into hospitals in Shenzhen. A possible similar migration of MRSA strains from the community into hospitals was reported for clone ST30-SCCmec IV-t019 which was the initially prevalent clone of

Table 5. Distribution of CC59-t437-SCCmec IV/V-*agr* I in CA-MRSA, HA-MRSA and HACO-MRSA strains

Category	CC (n)	ST (n)	<i>spa</i> (n)	SCCmec type (n)	<i>agr</i> type (n)	PFGE pattern (n)
CA-MRSA	59 (36)	59 (9)	t437 (9)	IV (8), V (1)	I (9)	USA1000 (9)
		59 (13)	t437 (13)	IV (6), V (7)	I (13)	Other (13)*
		338 (11)	t437 (11)	V (11)	I (11)	Other (11)
		2961 (1)	t437 (1)	V (1)	I (1)	Other (1)
		2962 (1)	t437 (1)	V (1)	I (1)	Other (1)
		3189 (1)	t437 (1)	IV (1)	I (1)	Other (1)
HA-MRSA	59 (13)	59 (7), 3031 (1)	t437 (8)	IV (8)	I (8)	USA1000 (8)
		338 (1)	t437 (1)	V (1)	I (1)	USA1000 (1)
		59 (1), 338 (2)	t437 (3)	V (3)	I (3)	Other (3)
		59 (1)	t437 (1)	IV (1)	I (1)	Other (1)
HACO-MRSA	59 (36)	59 (6)	t437 (6)	IV (4), V (2)	I (6)	USA1000 (6)
		3191 (1)	t437 (1)	IV (1)	I (1)	USA1000 (1)
		59 (17)	t437 (17)	IV (9), V (8)	I (17)	Other (17)
		338 (11)	t437 (11)	V (11)	I (11)	Other (11)
		2982 (1)	t437 (1)	IV (1)	I (1)	Other (1)

CC, Clonal complex; ST, sequence type; CA-MRSA, community-associated methicillin-resistant *Staphylococcus aureus*; HA, hospital-associated; HACO, healthcare-associated community-onset.

\* MRSA strains distinct from USA clonal type patterns.

CA-MRSA but was also found in HA-MRSA strains in the Philippines [25]. Such community-hospital transmission may have occurred through healthcare workers and possibly the environment of patients discharged from hospitals. This underlines the need for hospitals to implement routine screening of patients for MRSA colonization on admission to hospital, and undertake active surveillance for MRSA carriage in 'patient-facing' staff.

The globally predominant CA-MRSA clones are ST1-IV (WA-1, USA400), ST5-IV (Argentina), ST8-IV (USA300), ST30-IV (South West Pacific clone), ST59-V (Taiwan clone), ST72-IV (Korea clone) and ST80-IV (European clone) [26]. However, in our study, the lineages that were previously most commonly associated with CA-MRSA were found in each of the three categories. This reinforces our view that transmission events from community to hospital patients are important factors that can affect the composition of each category, which in turn may contribute to evolutionary changes in the molecular characteristics of dominant strains. Such a scenario occurred with SCCmec type III which was by far the most prevalent *mec* element (87.3%) in HA-MRSA strains isolated in the Chinese mainland between 2001 and 2010 [27], but our findings suggest that it is being replaced by traditional CA-MRSA SCCmec types IV/V. Such a change may contribute to increased fitness of strains in hospital environments.

PFGE typing showed that approximately one-fifth of MRSA study strains were USA1000, which was one of the major CA-MRSA clones circulating in Taiwan [28], while the second predominant type, USA400, is a traditional CA-MRSA clone [29]. This finding underlines their prevalence in communities and hospitals. It has been well documented that CC1 isolates correspond to USA400 and CC59 to USA1000 [30], but in our study USA400 was associated not only with CC1 ( $n=13$ ), but also CC59 ( $n=2$ ) and CC9 ( $n=1$ ), and USA1000 with CC59 ( $n=37$ ) and CC239 ( $n=1$ ). This further supports the view that the traditional relationship between CCs and PFGE clonal groups is undergoing gradual change. Additionally, across all infection categories, 63.9% of MRSA strains did not belong to USA1000, 1000 and 1100, which provides a diverse background to give a context for the proliferation of the predominant clone, CC59-t437-SCCmec IV/V-*agr*, I across Shenzhen hospitals.

Others have reported that intensive-care units (ICUs) are high-risk areas with a high level of endemic MRSA [31–33]. However, in our study, MRSA infections were commonly identified in departments of general surgery, mastitis and orthopaedics, and <5% of cases occurred in the ICU setting, suggesting that we may have a different clinical distribution of cases compared to some countries, and indicates a need to focus more on departments other than ICUs.

An apparently unique observation of this study was that HACO-MRSA strains were most commonly isolated from patients with mastitis. Of 20 such strains, 13 were isolated from pus samples from nursing mothers in a specialist maternity and child healthcare hospital. Nine of these strains were of CC59-t437-SCC*mec* IV/V-*agr* I but PFGE typing showed no relationship between these or others. The telephone survey confirmed that these mothers took at least 2–3 months to recover from the infection and this seriously affected breastfeeding. Such cases therefore have a high risk of transmitting infections to their babies, and it would seem useful to implement screening of oral MRSA colonization in nursing mothers' babies as an infection control measure in the future. Additionally, two HA-MRSA strains originated from mastitis cases who were not nursing mothers but molecular typing showed that the strains were CC59-t437-SCC*mec* V-*agr* I and CC59-t14776-SCC*mec* IV-*agr* I, which were similar to the strains isolated from the nursing mothers mentioned above.

Our study is subject to some limitations. First, there are more than 90 hospitals in Shenzhen; we only included nine sentinel hospitals which may not be fully representative of all MRSA patients in Shenzhen hospitals. Second, it is possible that not all doctors used the same criteria when requesting bacterial cultures, so some cases could have been missed. Third, although a total of 812 MRSA strains were obtained, only 183 strains were analysed due to exclusion of colonizing strains and specimens with incomplete information, which could also have biased the results.

In conclusion, in our sentinel hospital population, CA-, HA- and HACO-MRSA infections were caused predominantly by a common clone which exhibits combined characteristics of both CA-MRSA (CC59-t437-SCC*mec* IV/V) and HA-MRSA (*agr* I) lineages, but patients differed in demographic and clinical characteristics. This highlights the possible transmission of MRSA between community and hospitals. For MRSA prevention and control, hospitals should enhance surveillance in high-risk patients and healthcare personnel in clinical departments in addition to ICUs. Considering the high incidence of HACO-MRSA infections observed in nursing mothers, hospitals should implement oral MRSA screening of babies to stem mother-to-child transmission. Finally, continuous surveillance of the epidemiology and documentation of the changing aetiology of MRSA strains is necessary for the effective treatment of cases and control of transmission events.

## SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit <http://dx.doi.org/10.1017/S0950268816001552>.

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## DECLARATION OF INTEREST

None.

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