Sequence type 8 as an emerging clone of methicillin-resistant *Staphylococcus aureus* causing bloodstream infections in Taiwan

Pao-Yu Chen, Yu-Chung Chuang, Jann-Tay Wang, Wang-Huei Shenga, Yee-Chun Chen, and Shan-Chwen Chang

*Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan; Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan; National Institutes of Infectious Diseases and Vaccinology, National Health Research Institutes, Zhunan, Taiwan; College of Medicine, National Taiwan University, Taipei, Taiwan*

**ABSTRACT**

Sequence type (ST) 8 has not been a common methicillin-resistant *Staphylococcus aureus* (MRSA) clone in Asia until recently. We aimed to determine the clinical significance and microbiological characteristics of MRSA bacteraemia (MRSAB) caused by ST8 and other endemic clones. A total of 281 non-duplicated MRSAB were identified in a medical centre between 2016 and 2018. Sequencing of target genes was performed to determine ST and to confirm ST8 belonging to USA300. Antimicrobial susceptibility testing was performed by using Sensititre standard panel. In total, ST8 accounted for 18.5% of MRSAB ranking after ST239 (31.0%) and ST59 (23.5%). However, it increased to become the most prevalent clone finally. All ST8 isolates belonged to spa clonal complex008, and carried SCCmec IV/IVa, PVL and ACME genes, indicating USA300. ST8/USA300 isolates were highly susceptible to non-β-lactams antibiotics, except fluoroquinolone and erythromycin. ST8/USA300 MRSAB is commonly developed in community settings with either healthcare risks or not (71.2%). Compared to other STs MRSAB, ST8/USA300 MRSAB patients had more diabetes mellitus (50.0%), more admitted from long-term care facility residents (25.0%), had more skin ad soft tissue infection as primary focus (25.0%), and had fewer vascular devices (26.9%) at MRSAB onset. On multivariable analysis, isolates with vancomycin MIC were significantly associated with mortality in the dose–response relationship, rather than STs. This report depicts the clinical features of ST8/USA300 MRSAB and clonal shift from prior endemic clones to ST8/USA300. Our data strongly support long-term surveillance to ascertain whether ST8/USA300 will successfully disseminate and demonstrate its pathogenicity on clinical outcomes.

**ARTICLE HISTORY** Received 22 July 2021; Revised 24 August 2021; Accepted 12 September 2021

**KEYWORDS** USA300; multilocus sequence typing; spa typing; PVL; ACME

**Introduction**

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the important drug-resistant pathogens and causes high disease burdens globally [1, 2]. Efforts into infection control and implementation of antibiotic stewardship have seen the secural trends of the incidence of hospital-acquired (HA)-MRSA infections decrease, but community-acquired (CA)-MRSA infections are still prevalent worldwide, including Taiwan [3, 4].

Sequence type (ST) 8 has been the dominant clone causing CA-MRSA infections in both North and South America for two decades, while genotypes of CA-MRSA in Asia are diverse and country-specific. For example, ST59 was dominant in Taiwan and China, ST72 in South Korea, ST5 in Japan, and ST30 in southeast Asia, while ST80 is prevalent in the background of genetic heterogeneity in Europe [5–6]. Though clone differences of CA-MRSA around the world have been characterized, the predominant clinical presentations of these CA-MRSA clones are skin and soft tissue infections [5–6]. Additionally, these clones commonly carry staphylococcal cassette chromosome (SCC) mec types IV or V without antibiotic-resistant genes, except mecA [5, 6]. This genetic feature consequently leads to CA-MRSA being less resistant to non-β-lactams.

Clonal expansion of CA-MRSA clones and its subsequent introduction to the hospital environment were observed in the past decades. As intermixing of CA- and HA-MRSA clones, several specific CA-MRSA clones further replaced the original HA-MRSA clones to become prevalent in both communities and hospitals [3–6]. In the United States (USA), USA100 (ST5), the traditional HA-MRSA clone has been substituted by USA300 (ST8) [4–6], defined as possessing similar banding patterns by pulsed-field gel electrophoresis (PFGE) and having the following characteristics:
spa-clonal complex (CC)008, methicillin resistance, Panton-Valentine Leukocidin (PVL) and Arginine Catabolic Mobile Element (ACME)-encoding genes [7,8]. The dominance of ST8 is less reported in continents other than the USA, but ST8 has not uncommonly been identified in clinical specimens in Asia since 2010 [9,10]. Prior studies from Japan and South Korea demonstrated that ST8 spreads in both communities and hospitals to become an emerging clone causing MRSA infections, further confirming that ST8 belongs to USA300 under the aforementioned definitions [9,10].

In Taiwan, ST59 used to be the major CA-MRSA clone, while the proportions of ST59 in HA-MRSA bacteraemia (HA-MRSAB) increased as high as that of the HA-MRSA clone ST239 after 2010 [11]. Additionally, ST45 has emerged since 2010 with the highest carriage rates and environmental contamination of ST45 in long-term care facilities [12,13]. In parallel, one longitudinal multicentre study between 1995 and 2015 suggested ST8 is also emerging among MRSA colonization and infection isolates by showing most ST8 (85%) identified after 2010 [14]. A later study demonstrated ST8, in second place after ST59, accounted for 20% of the collected MRSA isolates causing cellulitis or osteomyelitis between 2016 and 2018 [15]. The findings that ST8 have abruptly increased in non-bloodstream invasive infections raises the question as to whether ST8 subsequently has become prevalent and replaced prior endemic MRSA clones causing bacteraemia in Taiwan. In addition, it is important to ascertain whether ST8 MRSA in Taiwan was genetically related to USA300.

In this study, we planned to test clonal shift to ST8 in the contemporary collection of MRSA bloodstream isolates, to infer the genetic relationship of ST8 and USA300, and further to compare clinical characteristics, antibiotic susceptibility testing and patient outcomes among ST8 and other STs in Taiwan.

Materials and methods

Patients

From 1 January 2016 to 31 December 2018, all adult patients age ≥20 years with MRSAB admitted to National Taiwan University Hospital (NTUH), a major tertiary teaching hospital with 2500 beds located in northern Taiwan, were retrospectively enrolled in this study. MRSAB was defined as ≥1 culture from a blood sample yielding MRSA with compatible symptoms/signs [16]. If a patient had multiple episodes of MRSAB during the study period, only the first episode was considered in the present study. This study was approved by the Institutional Review Board (IRB) at NTUH (NTUH-201701082RIND, NTUH-202102048RIND).

Definitions

A standardized case report form was used to collect the patients’ demographic, clinical, and routine laboratory data, including the patient’s age and sex, the primary foci of the bacteraemia, defined by the U.S. Centers for Disease Control and Prevention [17], presence of shock within 24 h of the onset of MRSAB, the underlying diseases and conditions, the Charlson morbidity index, and Pitt bacteraemia score at the time of onset of MRSAB. We epidemiologically categorized patients with MRSAB into three groups: hospital-acquired (HA; index culture obtained ≥48 h after admission); healthcare-associated, community onset (HACO; at least one healthcare-associated risk factor); and CA (community-associated). Healthcare-associated risk factors included hospitalization within one year before MRSAB, residence in a long-term care facility for any duration, surgery, dialysis, or presence of a central vascular catheter [16].

Microbiological methods

The MRSA blood isolates have been prospectively preserved in the research laboratory of the Department of Internal Medicine at NTUH for more than 40 years. All preserved and available bacterial isolates were re-identified and confirmed as S. aureus by Gram stain, catalase-activity tests, and coagulase latex agglutination tests. MRSA was identified using CHROMagarTM MRSA plates. In vitro susceptibilities to erythromycin, clindamycin, gentamicin, oxacillin, tetracycline, trimethoprim/sulfamethoxazole (SXT), rifampin, ciprofloxacin, vancomycin, linezolid, and daptomycin were determined by the broth microdilution method using the Sensititre standard panel GPALL1F. The susceptibility test results were interpreted using the criteria provided by the Clinical Laboratory Standards Institute [18].

Genomic DNA was extracted using a Blood and Tissue Genomic DNA Miniprep system kit (VIogene) according to the instructions of the manufacturer. Multilocus sequence typing (MLST) was performed as described previously [19]. In brief, the S. aureus MLST scheme consisted of the seven housekeeping genes, including arcC, aroE, glp, gmk, pta, tpi, and yqiL. Fragments of each gene were used for PCR amplification, followed by sequencing. To assign allele numbers and sequence types, the sequence results of each locus were compared to the S. aureus MLST database (https://pubmlst.org/). A combination of the allele numbers generated from the seven gene fragments yields unique STs, and new allele numbers and STs numbers would be submitted to the database.
The reference strain FPR3757, belonging to USA300 with multidrug resistance, was used as a standard for confirming whether a clinical MRSA isolate belonged to USA300. For those identified to be ST8 isolates, the presence of the SCCmec elements IV with subtyping IVa to IVd was determined by methods described previously [16,20], as well as the presence of PVL and ACME-encoding genes [16,21]. Analysis of the polymorphic X-region of the protein A gene (spa) and assignment of spa types were performed using the Spa typing plug-in tool of the BioNumerics software package (Applied Maths, Ghent, Belgium) [16]. Analysis of PFGE patterns was performed by using BioNumerics software (Applied Maths, Ghent, Belgium) [16]. Band position tolerance and optimization were set at 1.25 and 0.5%, respectively. Banding patterns within 80% similarity were considered the same pulsotype [7].

Results

A total of 281 patients were found to have their first episode of MRSAB during 2016–2018. Overall, ST239 (87, 31.0%) and ST59 (66, 23.5%) remained the most common two sequencing types (Table 1). ST8 initially accounted for 15% and later jumped to 25% (P for trend, 0.08), paralleling the declines of both ST239 and ST59 in 2018. ST8 finally became the most prevalent ST (Figure 1(A); Supplementary Table 1). Most ST8 MRSAB developed in the community (71.2%, consisting of 17 CA-MRSAB and 20 HACO-MRSAB). The distribution of STs among the three onset settings was quite unique (Figure 1(B)). ST8 was the most common ST in CA-MRSAB, instead of ST59. Meanwhile, ST239 remained dominant in HA-MRSAB, and ST59 was equally distributed in three onset settings. ST45 was nearly all found in HACO-MRSAB and HA-MRSAB.

As shown in Table 1, patients with ST8 MRSAB were more likely to have diabetes mellitus (50.0%), and to be from long-term care facilities (25.0%), while they were most unlikely to have a vascular device at the onset of MRSAB (26.9%). A higher proportion of patients with ST8 MRSAB had skin and soft tissue infections (SSTI) (25.0%), endocarditis (7.7%), and deep abscess (7.7%) as the primary focus, despite overall comparisons not meeting statistical significance. Seventy percent of MRSAB patients received effective anti-MRSA therapy within 48 h after onset of bacteremia, and glycopeptide was the most common drug (88.3%) as the first therapeutic agent without significant difference among different causative STs.

For the 52 ST8 isolates in the present cohort and the USA300 reference strain, FPR3757, the dendrogram via PFGE, SCCmec typing, and spa typing is shown in Figure 2. The overall similarities were 84.0% by the banding pattern of PFGE. All ST8 isolates carried SCCmec IVa, except one as SCCmec IV. Using spa typing, almost all (n = 48) were categorized as t008, and the others each included one of t121, t574, t2558, and t3081, clustered into spa-Clonal Complex008 (spa-CC008). All the 52 ST8 isolates were positive for both PVL and AMCE (Figure 2), and thus all of them were considered to belong to USA300.

As shown in Table 2, nearly all 281 isolates were susceptible to common parental anti-MRSA agents, including vancomycin, daptomycin and linezolid. Only two ST239 isolates were not susceptible to daptomycin. ST8 isolates were generally susceptible to other non-β-lactam antimicrobial agents, except for being resistant to moxifloxacin and erythromycin (resistance rates, 98.1% and 82.7%, respectively). ST8 isolates were also resistant to ciprofloxacin and levofloxacin (Figure 2), while FPR3757 demonstrated not only resistance to different quinolones and erythromycin, but also showed resistance to clindamycin and tetracycline. ST239 isolates showed its multi-drug resistant (MDR) nature, except for being susceptible to rifampin (94.3%). About half of ST59 isolates were resistant to gentamicin and tetracycline, and nearly all were resistant to clindamycin and erythromycin (Table 2). By pairwise comparisons, ST8 was significantly less susceptible to moxifloxacin compared to ST59, ST45, or other STs (all P < 0.001), except ST239.

As shown in Table 1, the overall mortality of patients with MRSAB was 40.2%. Rates of persistent bacteremia and in-hospital mortality among patients with ST8 MRSAB were significantly lower than those among patients with ST239 MRSAB (3.9% vs. 24.1%, and 30.8% vs. 56.3%, respectively) but equivalent to those among patients with ST59 MRSAB. Predictors

Statistics

Continuous variables were described as medians and interquartile, and compared by using the Kruskal–Wallis one-way analysis of variance (ANOVA) with the Mann–Whitney U test as the post-hoc analysis. Categorical variables were described as percentages and compared by using the chi-square test with Bonferroni-adjusted α for pair-wise comparisons as post-hoc analysis. The time trend of rates of ST8 was examined by a logistic regression analysis. The predictors for all-cause in-hospital mortality were identified using logistic regression models. All parameters were initially tested using univariable analysis, and those with p values of <0.10 or biologically relevant variables were considered in the multivariable analysis. A stepwise model comparison and Akaike’s information criterion were used to determine the best model for the analysis of the multiple variables. All statistical analyses were performed using Stata software (version 14; StataCorp, College Station, TX). Two-sided P values less than 0.05 were considered significant.
| Table 1. Comparisons of clinical characteristics and outcomes of 281 patients with methicillin-resistant *Staphylococcus aureus* bacteraemia by sequencing types. |
|---------------------------------|
| **Demographics**                |
| Age, y, median (IQR)            |
| Gender, Male                    |
| Underlying diseases or conditions |
| Cardiovascular diseases         |
| Respiratory diseases            |
| Neurology diseases              |
| Hepatobiliary diseases          |
| Chronic renal impairment        |
| Chronic dialysis                |
| Rheumatology diseases           |
| Diabetes mellitus               |
| Solid tumour                    |
| Hematological malignancy        |
| Charlson comorbidity index, median (IQR) |
| Long-term care facility         |
| Vascular device at onset        |
| Severity                        |
| Shock                           |
| Pitt bacteremia score, median (IQR) |
| Infection focus                 |
| Primary                         |
| Catheter-related                |
| Device-related                  |
| Skin and soft tissue            |
| Pleuropulmonary                  |
| Native ostearticular            |
| Endocarditis                    |
| Septic                          |
| Deep infection                  |
| Management                      |
| Effective antibiotics within 48 h after onset |
| Glycopeptide as the first agents |
| Source control                  |
| Infectious disease consultation |
| Outcomes                        |
| Persistent bacteremia > 7 days  |
| In-hospital mortality           |
| **P-value**                     |
| **Total**                       |
| **ST8**                         |
| **ST239**                      |
| **ST59**                       |
| **ST45**                       |
| **Other STs**                   |
| **Overall**                     |
| **ST8 vs. ST239**               |
| **ST8 vs. ST59**                |
| **ST8 vs. ST45**                |
| **ST8 vs. Other STs**           |
| **Abbreviations:** ST, sequence type; IQR, interquartile range. Data are presented as no. (%) unless otherwise indicated. ST3235 (2), ST72 (1), ST89 (1), ST789 (1), ST1232 (1), and non-typable (4). ST14 STs are identified in 47 isolates and listed as follows: ST30 (n = 14), ST5 (6), ST508 (4), ST338 (3), ST15 (2), ST188 (2), ST398 (2), ST753 (2), ST965 (2), ST3235 (2), ST72 (1), ST89 (1), ST89 (1), ST1232 (1), and non-typable (4). Pairwise comparisons with Bonferroni adjustment was performed if overall comparisons were statistically significant (P < 0.05), and only pairwise comparisons among ST8 vs. different STs were reported. The detailed statistics are shown in Supplementary Table 2. Bold type indicates P < 0.05. Vascular devices consisted of any type of central vascular catheters, vascular grafts, peripherally inserted central catheters, and any cardiac devices, including heart valves, implantable pacemakers, automatically implantable cardioverter-defibrillators, left ventricular assist devices, and extracorporeal membrane oxygenation. Device-related infections were defined as infections due to any devices other than vascular device, including prosthetic joints, orthostatic implants, biliary stents, urinary stents, draining tubes, percutaneous feeding tubes, intrathelial catheters, peritoneal dialysis catheters, and oesophageal stents. Pleuropulmonary infections included pneumonia, necrotizing pneumonia, lung abscess, and empyema. Deep infections were not related to surgery or prosthesis. Source control included removal of vascular catheters and foreign devices as well as procedures such as drainage of skin, deep or visceral abscesses, debridement of infected tissue, and operative joint irrigation and drainage. Pairwise comparison, P < 0.05: ST59 vs. other STs. No pairwise comparison showed statistical significance (P > 0.05). Pairwise comparison, P < 0.05: ST239 vs. ST59. |
associated with in-hospital mortality were further analysed by performing the logistic regression analysis (Table 3). In univariable analysis, HA-MRSAB, a vascular device at onset, shock, the Pitt bacteraemia score, SSTI, pleuropulmonary infection, ST239, and isolates with vancomycin MIC of 1 or 2 mg/L (MIC of 0.5 mg/L as reference) were significant predictors for mortality. In addition to shock (adjusted odds ratio [aOR], 3.16; 95% confidence interval [CI], 1.52–6.60), and the Pitt bacteraemia score (aOR, 1.26; 95% CI, 1.10–1.45), isolates with vancomycin MIC of 1 mg/L (aOR, 2.84; 95% CI, 1.01–7.98) and of 2 mg/L (aOR, 8.45; 95% CI, 2.08–34.38) were significantly associated with mortality in a dose-response manner by multivariable analysis. On the contrary, SSTI (aOR, 0.39; 95% CI, 0.18–0.87) was conversely associated with mortality.

**Discussion**

In Taiwan, ST59 and ST239 were long recognized as the dominant CA-MRSA and HA-MRSA clones, respectively, until this study [5,6,16]. Surprisingly, ST8 was not uncommon since 2016 but abruptly saw

![Figure 1. The change in the proportion of ST8, ST239, ST59, ST45, and other STs among 281 methicillin-resistant Staphylococcus aureus blood isolates by years (a) and by onset setting (b). Abbreviations are as follows: ST, sequence type; CA, community associated; HACO, healthcare-associated, community onset; HA, hospital acquired.](image-url)
a 1.5-fold increase to become the most prevalent ST in 2018. Further, all ST8 isolates in this report were clustered to USA300 by a consensus definition [7,8]. Our finding that ST8/USA300 was most dominant in CA-MRSA, followed by HACO-MRSAB and HA-MRSAB, mirrored the dissemination direction of USA300 in the USA and other USA300 endemic regions, suggesting clonal expansion from community to hospital [5,6]. As for the infection focus of MRSAB, ST8/USA300 had the highest proportions for SSTI, endocarditis and deep-seated infections among different STs. Meanwhile, ST8/USA300 generally exhibited susceptibilities to non-β-lactams other than fluoroquinolones.

In parallel, Peng et al. reported ST8 became the second most common clone, causing cellulitis and osteomyelitis in both community and hospital settings from another hospital in Taiwan between 2016 and 2018 [15], while colonization rates of ST8 MRSA varied in Taiwan. Our recent surveillance study targeting Healthy adults between 2017 and 2018 [22] demonstrated that the carriage rate of ST8 in Taiwan remained low (<1%), as well as one large-scale longitudinal study from 1995 to 2015, confirming ST8 belonged to USA300 by whole-genome sequencing (WGS) [14]. Another surveillance study, however, showed the carriage rate of ST8 was common (11.3%) in long-term care facilities in 2016 [13]. Whether these patterns simply represent local secular trends or different phases of clonal expansion and/or contraction is unclear [23]. Our finding in this report with others may suggest ST8/USA300 is expected to

Figure 2. Molecular characteristics of 52 ST8 methicillin-resistant *Staphylococcus aureus* blood isolates. Spa types t121, t574, t2558, and t3081 are variants of t008. Plus symbols indicate detection of a given gene, and black squares indicate resistance to a given antibiotic. Abbreviations are as follows: PFGE, pulsed-field gel electrophoresis; no., number; SCC*mec*, staphylococcal cassette chromosome *mec*; PVL, Pantone-Valentine leucocidin-encoding genes; ACME, arginine catabolic mobile element-encoding genes.
| Antibiotic   | Range (mg/L) | MIC<sub>50</sub>/MIC<sub>90</sub> (mg/L) | S, n (%)   |
|-------------|--------------|--------------------------------------|------------|
| Vancomycin  | 0.5–2        | 1/1                                  | 281 (100.0)|
|             | 0.5–1        | 1/1                                  | 52 (100.0)|
|             | 0.5–2        | 1/2                                  | 87 (100.0)|
|             | 0.5–1        | 1/1                                  | 66 (100.0)|
|             | 0.5–1        | 1/1                                  | 29 (100.0)|
|             | 0.5–1        | 1/1                                  | 47 (100.0)|
|             | 0.5–1        | >0.99                                | >0.99      |
| Daptomycin  | 0.5–4        | 0.5/0.5                              | 281 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 52 (100.0)|
|             | 0.5–4        | 0.5/0.5                              | 87 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 66 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 29 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 47 (100.0)|
|             | 0.5–0.5      | >0.99                                | >0.99      |
| Linezolid   | 1–4          | 2/2                                  | 281 (100.0)|
|             | 2–2          | 2/2                                  | 52 (100.0)|
|             | 1–2          | 2/2                                  | 87 (100.0)|
|             | 2–4          | 2/2                                  | 66 (100.0)|
|             | 2–4          | 2/2                                  | 29 (100.0)|
|             | 2–4          | 2/2                                  | 47 (100.0)|
|             | 2–4          | >0.99                                | >0.99      |
| Gentamicin  | 2–0.5        | 0.5/0.5                              | 281 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 52 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 87 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 66 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 29 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 47 (100.0)|
|             | 0.5–0.5      | >0.99                                | >0.99      |
| Rifampin    | 0.5–4        | 0.5/0.5                              | 281 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 52 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 87 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 66 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 29 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 47 (100.0)|
|             | 0.5–0.5      | >0.99                                | >0.99      |
| TMP/SMX     | 0.5–4        | 0.5/0.5                              | 281 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 52 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 87 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 66 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 29 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 47 (100.0)|
|             | 0.5–0.5      | >0.99                                | >0.99      |
| Tetracycline| 2–0.5        | 0.5/0.5                              | 281 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 52 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 87 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 66 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 29 (100.0)|
|             | 0.5–0.5      | 0.5/0.5                              | 47 (100.0)|
|             | 0.5–0.5      | >0.99                                | >0.99      |
| Moxifloxacin| 0.25–4       | 0.25/>4                              | 281 (100.0)|
|             | 0.25–4       | 0.25/>4                              | 52 (100.0)|
|             | 0.25–4       | 0.25/>4                              | 87 (100.0)|
|             | 0.25–4       | 0.25/>4                              | 66 (100.0)|
|             | 0.25–4       | 0.25/>4                              | 29 (100.0)|
|             | 0.25–4       | 0.25/>4                              | 47 (100.0)|
|             | 0.25–4       | >0.99                                | >0.99      |
| Clindamycin | 0.5–2        | 0.5–2                                | 281 (100.0)|
|             | 0.5–2        | 0.5–2                                | 52 (100.0)|
|             | 0.5–2        | 0.5–2                                | 87 (100.0)|
|             | 0.5–2        | 0.5–2                                | 66 (100.0)|
|             | 0.5–2        | 0.5–2                                | 29 (100.0)|
|             | 0.5–2        | 0.5–2                                | 47 (100.0)|
|             | 0.5–2        | >0.99                                | >0.99      |
| Erythromycin| 0.25–4       | 0.25/>4                              | 281 (100.0)|
|             | 0.25–4       | 0.25/>4                              | 52 (100.0)|
|             | 0.25–4       | 0.25/>4                              | 87 (100.0)|
|             | 0.25–4       | 0.25/>4                              | 66 (100.0)|
|             | 0.25–4       | 0.25/>4                              | 29 (100.0)|
|             | 0.25–4       | 0.25/>4                              | 47 (100.0)|
|             | 0.25–4       | >0.99                                | >0.99      |

<sup>a</sup>Of 47 isolates, 14 STs were identified and listed as follows: ST30 (n=14), ST5 (6), ST508 (4), ST338 (3), ST15 (2), ST188 (2), ST398 (2), ST753 (2), ST965 (2), ST3235 (2), ST72 (1), ST89 (1), ST789 (1), ST1232 (1), and non-typable (4).

<sup>b</sup>Only pairwise comparisons with Bonferroni adjustment among ST8 vs. different STs were reported if overall comparisons were statistically significant (P<0.05).

<sup>c</sup>For isolates that tested erythromycin resistant and clindamycin susceptible or intermediate, testing for inducible clindamycin resistance by broth microdilution was performed to determine susceptibility to clindamycin.
achieve dominance causing MRSA colonization and infections despite the pre-existence of diverse MRSA genetic background, such as ST59, in the community. Although ST8/USA300 is most prevalent in North/South America [5], its dominance in the rest of the world is to a lesser extent. This is the first report, to our best knowledge, demonstrating ST8/USA300 successfully replaces a prior long-lasting endemic CA-MRSA clone, ST59 in Taiwan, outside the USA.

The reasons for clonal replacement are complex in CA-MRSA evolutions. PVL, SCCmec IV, and ACME genes are unique to CA-MRSA in different genetic lineages, which may offer evolutionary fitness compared to other MRSA clones [5,23]. In Taiwan, ST59 is positive for PVL with either SCCmec IV or SCCmec V_T, mostly without ACME [15], showing similar virulence to USA300 through Agr, α-toxin, and PSMA [24]. Compared to the virulent genetic components of ST59, all ST8/USA300 isolates in the current study had ACME, indicating the role of ACME as the major fitness determinant associated with the current clonal replacement in Taiwan.

Alternatively, we also speculated the increased consumption of fluoroquinolone (FQ) as one of the driving forces to replace FQ sensitive MRSA clones (ST59) with FQ resistant clones (ST8/USA300). Taiwan’s national health insurance (NHI) database showed a reduction in FQ consumptions since 2008 due to
antibiotic restrictions in Taiwan [25]. However, a recent study by the National Health Research Institutes in Taiwan showed private drug consumption accounted for a certain amount in the community not counted in the NHI database, likely resulting in underestimation of the true FQ consumption amount [25]. Therefore, the FQ positive selective pressure might remain significant, which in turn facilitated the clonal shift from ST59 to ST8/USA300 in Taiwan.

In addition to the overall similarities of 52 ST8/USA300 isolates by PFGE above 80.0%, our study, using other molecular typing methods, illustrated microevolutions of ST8/USA300 MRSA have developed in Taiwan, as spa typing showed four isolates possessed spa CC008, instead of spa -008. One prior study by performing a whole-genome sequence (WGS) to demonstrate the existence of ST8 with local transmission in Taiwan since 2005 further supports our findings [14]. Additionally, the antibiotic resistance patterns of ST8/USA300 isolates in our present study were not totally the same as the USA300 reference strain, FPR3757. Read et al also reported changes in plasmid and prophage content and antibiotic resistance of ST8/USA300 MRSA between index infection isolates and subsequent colonization isolates within a person [26]. Both results may reflect a loss of some mobile genetic elements encoding antibiotic resistance during evolutions of ST8/USA300 MRSA.

The aforementioned findings, however, cannot preclude the possibilities of multiple introductions of ST8/USA300 causing the current epidemic in Taiwan, like dissemination of USA300 to other continents [27]. Alternatively, ST8 MSSA may coincidentally acquire SCCmec IVa or IV to become ST8 MRSA, then disseminate from community to hospital, but this is less likely, because previous studies have shown extremely low prevalence (<1%) of ST8 MSSAB in Taiwan [28,29]. Further epidemiological studies using WGS analysis are warranted to depict the evolutions and dissemination of ST8/USA300 MRSA from colonization to invasive infections in Taiwan over time.

The role of USA300 in the mortality of patients with MRSA B remains debatable, albeit animal data support increased virulence with USA300 [30]. In the earlier U.S. study, patients with USA300 MRSA were associated with younger age and more SSTI, and were, in turn, associated with lower mortalities than those infected by USA100 [31]. Later, after intermixing of USA300 and USA100, mortality did not significantly differ between these two clones [32] or become even higher in USA300 [33]. Our data suggests the current situation of MRSA B in Taiwan was more similar to the context of the initial report of USA300 in the USA; thus, the impact of MRSA genotypes on clinical outcomes might be confounded by patient ages and clinical syndromes. Further, our results echo prior results showing vancomycin MIC as a detrimental factor for in-hospital mortality in a dose-response manner with most patients having received glycopeptide as the first anti-MRSA agent in the treatment of MRSA B [34].

Nevertheless, several limitations should be noted in our study. First, this was a single-centre retrospective study. Our study was conducted in a relatively short period; thus, bias was inevitable and generalization should be cautious. However, the Taiwan Surveillance of Antimicrobial Resistance (TSAR) program, conducted biennially to collect different micro-organisms from medical centres and regional hospitals throughout Taiwan since 1998, also found the rates of ST8/USA300 increased from 25.0% in 2016 to 30.0% in 2018 paralleling with the declining rates of ST239 and ST59 among difference clinical specimens, including blood, pus/abscess, and respiratory specimens [35]. Other reports in Taiwan also found ST8/USA300 becoming common in superficial and deep MRSA infections in the same period [15]. All above findings shed light on the emergence of ST8/USA300 as a dominant MRSA clone to cause different clinical syndromes in Taiwan. Conversely, our data lacked several confounding factors, including a history of prior MRSA colonization, household clusters, or prior antibiotic exposure. All warrant further investigation to depict the evolution and dissemination of the current ST8/USA300 epidemic in Taiwan.

Collectively, our study found there was a clonal shift of MRSA from ST239 and ST59 to ST8/USA300, especially in community settings. The long-term trend of MRSA infections in the USA has illustrated ST8/USA300 is difficult to eradicate in the community, therefore, an important task for current MRSA research is to delineate effective strategies to prevent dissemination and colonization of ST8/USA300 MRSA in the community, and subsequently to reduce the burden of MRSA infections. Also, continuing surveillance of molecular epidemiology of MRSA in Asia, especially in Taiwan, is indicated to ascertain whether ST8/USA300 will successfully expand with long-term fitness and to trace its pathogenicity on patient outcomes.

**Author contributions**

J-TW conceived, organized and supervised the project and proofread the manuscript. P-YC collected and analysed the data and drafted the manuscript. Y-CC supervised statistical analysis. All authors reviewed, revised and approved the final version of the manuscript.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).
Funding

This work was supported by the Ministry of Science and Technology in Taiwan [grant number 106-2314-B-002-101-MY3] and by National Taiwan University Hospital [grant number NTUH.109-O15]. The funding source played no role in study design and conduct, data collection, analysis or interpretation, writing of the manuscript, or the decision to submit it for publication.

ORCID

Jann-Tay Wang http://orcid.org/0000-0002-0924-3469

References

[1] CDC. Antibiotic resistance threats in the United States, 2019. Atlanta (GA): U.S. Department of Health and Human Services, CDC; 2019.

[2] Tacconelli E, Carrara E, Savoldi A, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis. 2018 Mar;18(3):318–327.

[3] Lai CC, Lin SH, Sheng WH, et al. Decrease in the incidence of meticillin-resistant Staphylococcus aureus nosocomial bloodstream infections in Taiwan. Int J Antimicrob Agents. 2013 Jun;41(6):591–592.

[4] See I, Mu Y, Albrecht V, et al. Trends in incidence of meticillin-resistant Staphylococcus aureus bloodstream infections differ by strain type and healthcare exposure, United States, 2005–2013. Clin Infect Dis. 2020 Jan 1;70(1):19–25.

[5] Lakhundi S, Zhang K. Methicillin-resistant Staphylococcus aureus: molecular characterization, evolution, and epidemiology. Clin Microbiol Rev. 2018 Oct;31(4):e00020–18.

[6] Turner NA, Sharma-Kuinkel BK, Maskarinec SA, et al. Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research. Nat Rev Microbiol. 2019 Apr;17(4):203–218.

[7] McDougall I, Steward CD, Killgore GE, et al. Pulsed-field gel electrophoresis typing of oxacillin-resistant Staphylococcus aureus isolates from the United States: establishing a national database. J Clin Microbiol. 2003 Nov;41(11):5113–5120.

[8] David MZ, Taylor A, Lynfield R, et al. Comparing pulsed-field gel electrophoresis with multilocus sequence typing, spa typing, staphylococcal cassette chromosome mec (SCCmec) typing, and PCR for pan-valentine leukocidin, arca, and opp3 in meticillin-resistant Staphylococcus aureus isolates at a U.S. medical center. J Clin Microbiol. 2013 Mar;51(3):814–819.

[9] Jung J, Song EH, Park SY, et al. Emergence of Panton-Valentine leukocidin-positive ST8-meticillin-resistant Staphylococcus aureus (USA300 clone) in Korea causing healthcare-associated and hospital-acquired bacteremia. Eur J Clin Microbiol Infect Dis. 2016 Aug;35(8):1323–1329.

[10] Takadama S, Nakaminami H, Sato A, et al. Dissemination of Panton-Valentine leukocidin-positive meticillin-resistant Staphylococcus aureus USA300 clone in multiple hospitals in Tokyo, Japan. Clin Microbiol Infect. 2018 Nov;24(11):1211 e1–1211 e7.

[11] Wang JT, Hsu LY, Lauderdale TL, et al. Comparison of outcomes among adult patients with nosocomial bacteremia caused by meticillin-susceptible and meticillin-resistant Staphylococcus aureus: A retrospective cohort study. PLoS One. 2015;10(12): e0144710.

[12] Tsao FY, Kou HW, Huang YC. Dissemination of meticillin-resistant Staphylococcus aureus sequence type 45 among nursing home residents and staff in Taiwan. Clin Microbiol Infect. 2015 May;21(5):451–458.

[13] Liu CY, Lai CC, Chiang HT, et al. Predominance of meticillin-resistant Staphylococcus aureus in the residents and environments of long-term care facilities in Taiwan. J Microbiol Immunol Infect. 2019 Feb;52(1):62–74.

[14] Huang YC, Chen CJ, Kuo CC, et al. Emergence, transmission and phylogeny of meticillin-resistant Staphylococcus aureus sequence type 8 (USA300) in Taiwan. J Hosp Infect. 2018 Nov;100(3):355–358.

[15] Peng KT, Huang TY, Chiang YC, et al. Comparison of meticillin-resistant Staphylococcus aureus isolates from cellulitis and from osteomyelitis in a Taiwan hospital, 2016–2018. J Clin Med. 2019 Jun;7(6):816.

[16] Wang JL, Wang JT, Chen SY, et al. Distribution of staphylococcal cassette chromosome mec types and correlation with comorbidity and infection type in patients with MRSA bacteremia. PLoS One. 2010 Mar 5;5(3):e9489.

[17] Horan TG, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008 Jun;36(5):309–332.

[18] CLSI. Performance Standards for Antimicrobial susceptibility testing. 29th ed. CLSI supplement M100S. Wayne (PA): Clinical and Laboratory Standards Institute; 2019.

[19] Enright MC, Day NP, Davies CE, et al. Multilocus sequence typing for characterization of meticillin-resistant and meticillin-susceptible clones of Staphylococcus aureus. J Clin Microbiol. 2000 Mar;38(3):1008–1015.

[20] Zhang K, McClure JA, Elsayed S, et al. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in meticillin-resistant Staphylococcus aureus. J Clin Microbiol. 2005 Oct;43(10):5026–5033.

[21] Berlon NR, Qi R, Sharma-Kuinkel BK, et al. Clinical MRSA isolates from skin and soft tissue infections show increased in vitro production of phenol soluble modulins. J Infect. 2015 Oct;71(4):447–457.

[22] Huang YS, Lai LC, Chen YA, et al. Colonization With multidrug-resistant organisms Among healthy adults in the community setting: prevalence, risk factors, and composition of Gut microbiome. Front Microbiol. 2020;11:1402.

[23] Planet PJ. Life after USA300: The rise and fall of a superbug. J Infect Dis. 2017 Feb 15 ;215(suppl_1):S71–S77.

[24] Chen CJ, Unger C, Hoffmann W, et al. Characterization and comparison of 2 distinct epidemic community-associated meticillin-resistant Staphylococcus aureus clones of ST59 lineage. PLoS One. 2013;8(9):e63210.

[25] Kuo SC, Shih SM, Hsieh LY, et al. Antibiotic restriction policy paradoxically increased private drug consumptions outside Taiwan’s National health...
[26] Read TD, Petit RA, 3rd, Yin Z, et al. USA300 Staphylococcus aureus persists on multiple body sites following an infection. BMC Microbiol. 2018 Dec 5;18(1):206.

[27] Toleman MS, Reuter S, Coll F, et al. Systematic surveillance detects multiple silent introductions and household transmission of methicillin-resistant Staphylococcus aureus USA300 in the east of England. J Infect Dis. 2016 Aug 1;214(3):447–453.

[28] Chen PY, Chuang YC, Wang JT, et al. Impact of prior healthcare-associated exposure on clinical and molecular characterization of methicillin-susceptible Staphylococcus aureus bacteremia: results from a retrospective cohort study. Medicine (Baltimore). 2015 Feb;94(5):e474.

[29] Chen YJ, Chen PA, Chen CJ, et al. Molecular characteristics and clinical features of pediatric methicillin-susceptible Staphylococcus aureus infection in a medical center in northern Taiwan. BMC Infect Dis. 2019 May 10;19(1):402.

[30] Li M, Diep BA, Villaruz AE, et al. Evolution of virulence in epidemic community-associated methicillin-resistant Staphylococcus aureus. Proc Natl Acad Sci U S A. 2009 Apr 7;106(14):5883–5888.

[31] Seybold U, Kourbatova EV, Johnson JG, et al. Emergence of community-associated methicillin-resistant Staphylococcus aureus USA300 genotype as a major cause of health care-associated blood stream infections. Clin Infect Dis. 2006 Mar 1;42(5):647–656.

[32] Simor AE, Pelude L, Golding G, et al. Determinants of outcome in hospitalized patients with methicillin-resistant Staphylococcus aureus bloodstream infection: results from national surveillance in Canada, 2008–2012. Infect Control Hosp Epidemiol. 2016 Apr;37(4):390–397.

[33] Kempker RR, Farley MM, Ladson JL, et al. Association of methicillin-resistant Staphylococcus aureus (MRSA) USA300 genotype with mortality in MRSA bacteremia. J Infect. 2010 Nov;61(5):372–381.

[34] van Hal SJ, Lodise TP, Paterson DL. The clinical significance of vancomycin minimum inhibitory concentration in Staphylococcus aureus infections: a systematic review and meta-analysis. Clin Infect Dis. 2012 Mar;54(6):755–771.

[35] Lauderdale TL. Taiwan Surveillance of Antimicrobial Resistance (TSAR): Gram-positive bacteria. 2019 Microbial Infectious Reference Laboratory (MIRL) Symposium-Antimicrobial Resistance in Hospitals and Community. October 18, 2019. Taipei.