

mecA-Positive *Staphylococcus aureus* with Low-Level Oxacillin MIC in Taiwan

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Although the presence of *mecA* is the genotypic determinant of methicillin-resistant *Staphylococcus aureus* (MRSA), certain MRSA strains, especially community-associated MRSA (C-MRSA), can display an oxacillin MIC in the Clinical and Laboratory Standards Institute susceptible breakpoint range (≤ 2 $\mu\text{g/ml}$). Among 91 and 180 isolates thought to be methicillin-susceptible *S. aureus* (MSSA) with oxacillin MICs of 2 and 1 $\mu\text{g/ml}$ as determined by the Sensititre broth microdilution test initially, 52 (57.1%) and 6 (3.3%), respectively, were *mecA* positive. These *mecA*-positive low-oxacillin-MIC isolates belong to the dominant Taiwan C-MRSA clone (clonal complex [CC] 59), 56 of which carried SCC*mec* type V and were *pvl* positive, and 43 of which belonged to *spa* CC t437. All 271 isolates were retested by Sensititre, as well as by Vitek II and disk diffusion (DD). Based on the oxacillin results, the sensitivities of the Sensititre, Vitek II, and DD methods were 48.3% (28/58), 46.6% (27/58), and 89.6% (52/58), respectively. Although cefoxitin was better at detecting these isolates, 12.1, 10.4, and 5.2% of these isolates were still misidentified as MSSA by Sensititre, Vitek II, and DD, respectively. These results highlight the difficulty in the accurate identification of MRSA with borderline oxacillin MICs in the CC59:SCC*mec* V clone, which likely has contributed to its spread in the health care and community settings. Since this clone has now been detected in other countries, and since other C-MRSA lineages have also been found to have low-level β -lactam resistance, the findings of the present study may be relevant to other regions. Further studies are warranted to determine the extent and clinical impact of such misidentification.

Methicillin-resistant *Staphylococcus aureus* (MRSA) arises when methicillin-susceptible *S. aureus* (MSSA) acquires the staphylococcal chromosome cassette *mec* (SCC*mec*) element, which contains the *mecA* gene encoding an altered penicillin-binding protein, PBP2' (PBP2a) with lowered binding affinity for β -lactams (12). The emergence of community-associated MRSA (C-MRSA) infections in the late 1990s has changed the molecular epidemiology of MRSA worldwide (31). Several reports have indicated C-MRSAs now predominate in the health care setting as the cause of health care-associated MRSA (H-MRSA) infections as well (3, 16, 22, 25).

Why certain clones of C-MRSAs were able to establish a niche and spread rapidly in different geographic regions is unclear. Compared to H-MRSAs, C-MRSAs are less multidrug resistant to non- β -lactam agents, and some C-MRSAs display low-level oxacillin resistance (28, 30). ST59, the founder of clonal complex 59 (CC59), is prevalent in Taiwan (5, 14), and comprises two types: the predominant one carries SCC*mec* type V and is *pvl* positive, while the *pvl*-negative ST59 strains mostly carry SCC*mec* type IV (1, 5, 14). In addition to being the dominant C-MRSA in Taiwan, the CC59:SCC*mec* V clone has also been a significant cause of hospital-acquired bloodstream infections in a large medical center in Taiwan (4).

In addition to being highly prevalent in Taiwan, the ST59:SCC*mec* V "Taiwan clone" was recently reported as the most common CC59 C-MRSA in Western Australia (7). This clone also accounted for 13.1% of C-MRSA isolated from Chinese children in multiple hospitals in China (10). In Europe, the ST59:SCC*mec* V MRSA has also been detected in the Netherlands (15), while SCC*mec* V carrying ST338, a single-locus variant of ST59, has also been found in Poland (19). These reports indicate the potential for this clone to spread in other countries.

Accurate identification of methicillin (oxacillin) resistance in

S. aureus is important for treatment and infection control purposes. Previously, while investigating genotypes of MSSA, we discovered several ST59 isolates thought to be MSSA with oxacillin MICs in the susceptible range (1 to 2 $\mu\text{g/ml}$) but contained type V SCC*mec* (4). The present study was carried out to compare methods for MRSA detection using a retrospective analysis of *S. aureus* isolates with oxacillin MICs of 1 to 2 $\mu\text{g/ml}$ obtained through our national surveillance program. Our results confirmed that the *pvl*-positive ST59:SCC*mec* V clone is the major contributor of this low-level oxacillin-resistant *mecA*-positive phenotype. We also found fluctuation in the performance of the commonly used susceptibility testing methods in detecting this particular clone of MRSA.

MATERIALS AND METHODS

***S. aureus* isolates.** All *S. aureus* isolates from the Taiwan Surveillance of Antimicrobial Resistance (TSAR) program from years 2002, 2004, 2006, and 2008 with oxacillin MICs of 2 $\mu\text{g/ml}$ were included in the present study. Isolates with oxacillin MICs of 1 $\mu\text{g/ml}$ were also randomly selected for the study to include one to four isolates from each hospital (if present) each year. TSAR is a biennial national surveillance program of multiple hospitals throughout Taiwan (20). During July and September of the collection year, each hospital first collected 200 consecutive isolates to include 50 outpatient and 150 inpatient (30 intensive care unit [ICU] patients, 100 non-ICU adults, and 20 pediatric patients) isolates, after which

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20 (2002 to 2006) to 50 (2008) isolates from blood and other sterile body sites were collected. In addition to the specimen source, the hospitals also provided the patient age and duplicate isolates were excluded. The same hospitals participated in TSAR between 2002 and 2008 except TSAR V (2006), in which one hospital did not participate. These hospitals included 11 medical centers and 15 regional hospitals located throughout Taiwan. All isolates were stored at -80°C . The species identification and purity of the isolates were confirmed as described previously (20).

Antimicrobial susceptibility testing (AST). The reference broth microdilution (BMD) method was used for routine TSAR surveillance study. For isolates in 2002, 2004, and 2006, MICs of oxacillin and non- β -lactams were determined by the BMD method according to Clinical and Laboratory Standards Institute (CLSI) guidelines using custom-designed Sensititre panels (Trek Diagnostics, West Essex, England). Prior to 2006, only oxacillin MIC was used to determine methicillin resistance in staphylococci. For isolates in 2008, the Sensititre standard panel GPALL1F was used, which contained oxacillin and cefoxitin (6). For isolates between 2002 and 2006, an inoculum of 5×10^5 CFU/ml was used. However, for the GPALL1F panel, an inoculum of 3×10^5 CFU/ml was used according to the recommendation of the manufacturer. For the present study, 180 isolates with oxacillin MICs of 1 $\mu\text{g}/\text{ml}$ and all 91 isolates with oxacillin MICs of 2 $\mu\text{g}/\text{ml}$ regardless of *mecA* PCR results were tested by oxacillin and cefoxitin disk diffusion (DD) test, as well as by Vitek 2 AST-P583 according to the instructions of the manufacturer (bioMérieux, Marcy l'Etoile, France). These 271 isolates were also retested by broth microdilution method using the Sensititre standard panel GPALL1F. The Etest was performed on all *mecA*-positive isolates only. Test isolates were subcultured from -80°C twice prior to susceptibility testing. The BMD, Etest, and DD test samples were incubated at 35°C ; the cefoxitin results were read after 18 to 20 h of incubation, and the oxacillin results were read after 24 h of incubation (6).

Molecular typing. All isolates were first confirmed to be *S. aureus* by coagulase PCR (29). The *mecA* gene was determined by PCR by using the primers mA1 and mA2 according to a previously published protocol (17). SCC*mec* type was determined on all *mecA*-positive isolates. The genetic profiles of the *mecA*-positive strains were also determined based on protein A (*spa*) typing and Panton-Valentine leukocidin (*pvl*) gene detection. Multilocus sequence typing (MLST) was performed on strains selected from different *spa* types from each year. The protocols for MLST, *pvl* detection, *spa* typing, and SCC*mec* typing have been described previously (5, 8, 11, 17, 21).

Statistical analysis. Susceptibility interpretation analysis was made using the Whonet software (<http://www.who.int/drugresistance/whonetsoftware/en/>). Univariate analysis was performed with the Statistical Package for the Social Sciences version 18.0 (SPSS, Chicago, IL). A *P* value of <0.05 was considered statistically significant.

RESULTS

Totals of 864, 798, 694, and 804 nonduplicate *S. aureus* isolates were tested in 2002, 2004, 2006, and 2008, respectively, by the BMD method using the Sensititre panel. The oxacillin MIC distribution by year is shown in Fig. 1. There were 180, 130, 100, and 31 isolates with oxacillin MICs of 1.0 $\mu\text{g}/\text{ml}$ (OXA 1.0), while 34, 30, 13, and 14 isolates had oxacillin MICs of 2.0 $\mu\text{g}/\text{ml}$ (OXA 2.0) in 2002, 2004, 2006, and 2008, respectively. Together, these OXA 1.0 and OXA 2.0 isolates represented 14.0% (441/3,160) and 2.9% (91/3,160) of the total 3,160 *S. aureus* isolates tested.

All 91 OXA 2.0 isolates and 180 (40.8%) of the 441 randomly selected OXA 1.0 isolates were tested for the presence of *mecA* by PCR. Six of the OXA 1.0 isolates (3.3%, 6/180), while 52 (57.1%, 52/91) of the OXA 2.0 isolates tested *mecA* positive, and they were detected in all four study years (Table 1). A higher proportion of OXA 2.0 isolates from 2002 to 2004 were *mecA* positive compared to those from 2006 to 2008, but the difference

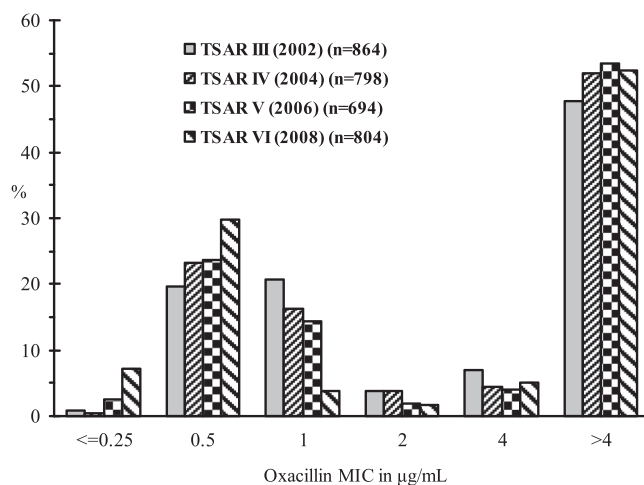


FIG 1 Oxacillin MIC distribution for *S. aureus* from the Taiwan Surveillance of Antimicrobial Resistance (TSAR) tested between 2002 and 2008 based on data from the original testing results by broth microdilution method used for routine surveillance study.

was not statistically significant (59.4% [38/64] versus 51.8% [14/34]; $P > 0.05$). Among these 58 *mecA*-positive, low-oxacillin-MIC isolates, 56 carried SCC*mec* type V, the other 2 carried SCC*mec* type IV (Table 1). The majority ($n = 43$ [74.1%]) of the isolates belonged to *spa* clonal complex t437. MLST was performed on 16 strains, and all of them belonged to clonal complex 59 (13 ST59 and 3 ST338).

Among the 271 isolates studied, 158 (58.3%) were from abscesses or wounds, and 43 (15.9%), 38 (14.0%), and 9 (3.3%) were from blood, respiratory, and urine specimens, respectively, with the remaining 23 (8.5%) from various other specimen types. Sixty isolates (22.1%) were from pediatric patients (≤ 18 years old), 204 (75.3%) were from adults, and the patient ages for the other 7 isolates are unknown. The 58 *mecA*-positive isolates were from 23 different hospitals in Taiwan; 54 (93.1%) were from wound specimens, 2 (3.4%) were from blood, 1 was from an ear, and the specimen source of one was unknown. The age of the patient was known for 55 of these isolates and included 19 (32.8%) pediatric and 36 (62.1%) adult patients. Twenty-six of the isolates were from inpatients, including 2 ICU and 24 non-ICU patients, and 32 were from outpatients. Thus, isolates from abscess or wound (odds ratio [OR], 11.11; 95% confidence interval [CI], 4.273 to 28.884; $P < 0.001$) and pediatric patients (OR, 2.163; 95% CI, 1.126 to 4.152, $P = 0.02$) were significantly more likely to be *mecA* positive with a low oxacillin MIC.

The performance of Sensititre (GPALL1F), Vitek II (AST-P583), and DD is summarized in the results presented in Table 2. Cefoxitin detected 87.9% ($n = 51$) of the 58 *mecA*-positive, low-oxacillin-MIC strains as MRSA by the Sensititre GPALL1F panel, while Vitek II detected 89.6% ($n = 52$). In contrast, by oxacillin MIC, only 48.3% ($n = 28$) of the Sensititre GPALL1F results and 46.6% ($n = 27$) of Vitek II results were in the resistant range (Table 2), and 19 isolates were missed by both methods (data not shown). Oxacillin and cefoxitin DD detected more isolates as MRSA: 89.6% ($n = 52$) and 94.8% ($n = 55$), respectively. However, among the 213 *mecA*-negative isolates, false-positive results by cefoxitin occurred in 1, 11, and 14 isolates by Sensititre, Vitek II, and DD, respectively. In addition, based on oxacillin results, 6

TABLE 1 Presence of *mecA* in *S. aureus* with oxacillin MICs of 1 and 2 µg/ml as determined by broth microdilution and SCC*mec* type of *mecA*-positive isolates

Yr	Oxacillin MIC of 1.0 µg/ml ^a			Oxacillin MIC of 2.0 µg/ml ^b		SCC <i>mec</i> type(s) of <i>mecA</i> -positive isolates (no. of isolates) ^c
	No of isolates	No. of isolates tested for <i>mecA</i>	No. (%) of <i>mecA</i> -positive isolates	No. of isolates tested	No. (%) of <i>mecA</i> -positive isolates	
2002	180	82	2 (2.4)	34	20 (58.8)	IV (1), V (21)
2004	130	50	1 (2.0)	30	18 (60.0)	V (19)
2006	100	35	1 (2.9)	13	7 (53.8)	IV (1), V (7)
2008	31	13	2 (15.4)	14	7 (50.0)	V (9)
Total	441	180	6 (3.3)	91	52 (57.1)	IV (2), V (56)

^a Due to the large number of isolates with an oxacillin MIC of 1.0 µg/ml (*n* = 441), only a portion (40.8%, 180/441) of randomly selected isolates were screened for the presence of *mecA*.

^b All isolates with an oxacillin MIC of 2 µg/ml were screened for the presence of *mecA*.

^c MLST was performed on 16 selected strains. Thirteen were ST59 and three were ST338, which is a single-locus variant of ST59.

and 40 of the *mecA*-negative isolates were considered methicillin resistant by Vitek II and DD, respectively. Therefore, although oxacillin DD had higher sensitivity, it had the lowest specificity (81.2%) and PPV (56.5%) compared to Sensititre and Vitek II. By Etest, which was performed only on *mecA*-positive isolates, 93.1% (*n* = 54) and 65.5% (*n* = 38) of the isolates would be considered MRSA, but those included 17 isolates with oxacillin MIC 3.0 µg/ml (data not shown).

DISCUSSION

The factors contributing to the dissemination of C-MRSA are still unclear. However, low-level and heterogeneous oxacillin resistance has been reported as a main characteristic of C-MRSA (23, 28, 30). The present study found a high rate of *mecA*-positive strains in *S. aureus* isolates with oxacillin MIC in the susceptible range of the CLSI breakpoint (<2 µg/ml), including those with oxacillin MICs of 1 µg/ml (3.3%, 6/180), and especially in those with oxacillin MICs of 2 µg/ml (57.1%, 52/91). Nearly all of these isolates belong to the prevalent C-MRSA clone, CC59:SCC*mec* V:*pvl*-positive, in Taiwan (5, 14). Although the majority were from wound specimens, these isolates were from different years and adult and pediatric patients in more than 20 hospitals, indicating that they were not the result of an outbreak.

Several studies have found cefoxitin to be superior to oxacillin in detecting *mecA*-mediated resistance, especially in *S. aureus* strains with low-level resistance (9, 24, 26, 27), and cefoxitin MIC was added as a screening method for determining *mecA*-mediated

resistance by the CLSI in 2008 (6). In the present study, without cefoxitin, both Sensititre and Vitek II would have missed more than half (51.7 to 53.4%) of these *mecA*-positive isolates because of their low oxacillin MICs. Our results confirm the importance of using cefoxitin as a marker for the detection of *mecA*-positive *S. aureus* and provided the genetic background for these isolates in our region. However, it is important to point out that even cefoxitin failed to identify several of these *mecA*-positive isolates (Sensititre missed 12.1% and Vitek II missed 10.4%) as MRSA, showing the difficulty in accurate identification of MRSA with borderline oxacillin MICs in the CC59:SCC*mec* V clone.

In the present study, upon retesting of the *mecA*-positive isolates with oxacillin MICs of 1 to 2 µg/ml by the broth microdilution method using the Sensititre standard GPALL1F panel, 28 of the 58 isolates had oxacillin MICs of >4 µg/ml, of which only 16 had oxacillin MICs of >4 µg/ml by Vitek II (data not shown). These results indicated fluctuation of the oxacillin MIC around the CLSI breakpoints; thus, oxacillin MIC is not a suitable method for detecting these *mecA*-positive isolates.

The reasons for the low-level oxacillin MIC in the CC59 genetic background are unknown at present. Previous studies have found *VraSR* and *PBP4* to be associated with oxacillin resistance in C-MRSA of other lineages (18). Chromosomal factors affecting the expression of PBPs other than *PBP2a*, and several auxiliary genes affecting cell wall synthesis may also be involved (2, 18). If these *mecA*-positive low-oxacillin MIC isolates were misidentified as MSSA initially, they may emerge as highly oxacillin-resistant

TABLE 2 Performance of Sensititre, Vitek II, and oxacillin and cefoxitin disk diffusion in detecting *mecA*-positive, low-oxacillin-MIC *S. aureus* isolates^a

Parameter ^b	Values (%) for all isolates (<i>n</i> = 271; <i>mecA</i> -positive isolates, <i>n</i> = 58; <i>mecA</i> -negative isolates, <i>n</i> = 213)					
	Sensititre broth microdilution ^c		Vitek II		Disk diffusion	
	Oxacillin	Cefoxitin	Oxacillin	Cefoxitin	Oxacillin	Cefoxitin
Sensitivity	48.3 (28/58)	87.9 (51/58)	46.6 (27/58)	89.6 (52/58)	89.6 (52/58)	94.8 (55/58)
Specificity	100 (213/213)	99.5 (212/213)	97.2 (207/213)	94.8 (202/213)	81.2 (173/213)	93.4 (199/213)
PPV	100 (28/28)	98.1 (51/52)	81.8 (27/33)	82.5 (52/63)	56.5 (52/92)	79.7 (55/69)
NPV	87.6 (213/243)	96.8 (212/219)	87.0 (207/238)	97.1 (202/208)	96.6 (173/179)	98.5 (199/202)

^a A total of 271 isolates were tested. These isolates tested as methicillin susceptible initially by Sensititre broth microdilution between 2002 and 2008, with oxacillin MICs of 1 (*n* = 180) or 2 (*n* = 91) µg/ml (see Table 1).

^b PPV, positive predictive value; NPV, negative predictive value.

^c Sensititre results are based on retest on GPALL1F panels containing cefoxitin and oxacillin.

strains upon subsequent exposure to β -lactam agents (2). Further studies are warranted to determine the extent and clinical impact of such misidentification. In addition, since these isolates were from both inpatients and outpatients, they may have been missed by infection control measures.

In conclusion, there exists a subpopulation of *mecA*-positive MRSA in Taiwan that can escape detection by the commonly used susceptibility testing methods especially if cefoxitin was not included in the routine panel. These isolates belong to the dominant Taiwan C-MRSA clones (CC59:SCC*mec* V:*pvl*-positive and *spa* CC t437). Since CC59:SCC*mec* V isolates have now been reported in other countries (7, 10, 13, 15, 19) and since other C-MRSA lineages have also been found to have low-level β -lactam resistance (23, 28, 30), the findings and implications of our study may be relevant to predominant C-MRSA lineages on other continents. Our results indicate that as more clinical laboratories switch to using automated susceptibility testing instruments for determining antimicrobial susceptibility, there is a need for careful evaluation of the performance of these methods for the detection of *mecA*-positive *S. aureus* with a low-level oxacillin resistance to prevent their further spread in health care and community settings.

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