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Evaluation of disc diffusion tests and agar screening for predicting *mecA*-mediated oxacillin resistance in *Staphylococcus lugdunensis* revealed a cefoxitin-susceptible, *mecA*-positive *S. lugdunensis* clonal complex 27 clone

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ABSTRACT

Objectives: This study evaluated disc diffusion tests and agar screening for detecting *mecA*-mediated oxacillin resistance in *Staphylococcus lugdunensis* (*S. lugdunensis*).
Methods: Staphylococcus lugdunensis isolates (n = 179) from diverse sources in Hong Kong during 1998–2018 were investigated by disc diffusion tests (cefoxitin and oxacillin) and inoculation onto oxacillin (1 μg/mL and 2 μg/mL) and chromID methicillin-resistant *Staphylococcus aureus* (MRSA) agars. The results were compared with *mecA* PCR as the reference. Isolates with discordant results were further tested by MIC and penicillin-binding protein 2a (PBP2a) assays.
Results: Cefoxitin and oxacillin zone diameters were not distributed in ways that allowed reliable division of the *mecA*-positive (n = 52) and *mecA*-negative (n = 127) isolates. On applying the 2019 Clinical Laboratory Standards Institute (CLSI) M100 breakpoints for cefoxitin disc results, there was 88% categorical agreement (CA) and 40% very major error (VME). Screening using 2 μg/mL oxacillin agar reliably differentiated *mecA*-positive and *mecA*-negative isolates (100% CA) without any major error (ME) or VME results. The performance of screening using 1 μg/mL oxacillin agar or ChromID MRSA agar was variable (74–89% CA, 0–38% ME and 0–37% VME). The *mecA*-positive isolates (n = 21) that could not be detected by the cefoxitin disc test were further characterised. The cefoxitin MIC for all 21 isolates was ≤4 μg/mL. Twenty isolates had an oxacillin MIC of 1–2 μg/mL and one had an oxacillin MIC of 4 μg/mL. All had positive PBP2a results and were typed as clonal cluster 27/SCC*mec* V.
Conclusions: These findings highlight the need to evaluate phenotypic methods using *mecA*-positive *S. lugdunensis* with different oxacillin resistance phenotypes.
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1. Introduction

In the recent years, oxacillin-resistant *Staphylococcus lugdunensis* (*S. lugdunensis*) (ORSL) has increasingly been recognised to cause community-associated and healthcare-associated infection and associated with the acquisition of SCC*mec* IV or V elements and clonal spread [1,2]. In Taiwan, among hospitals in the central and northern areas, several studies have identified a major endemic clone of sequence type (ST) 6 *S. lugdunensis* carrying SCC*mec* type V [2,3]. In Hong Kong, previous work has shown that emerging oxacillin resistance in *S. lugdunensis* in the hospitals is linked to the expansion of an ST3 clone carrying SCC*mec* V [1]. Although the ST3/SCC*mec* V clone was mainly identified among patients with end-stage renal failure, it has also been found to cause sporadic infections in other clinical settings [4].

Currently, the same set of MIC (cefoxitin and oxacillin) and disc (cefoxitin) breakpoints are recommended by the Clinical and Laboratory Standards Institute (CLSI) for *Staphylococcus aureus* (*S. aureus*) and *S. lugdunensis* [5]. In *S. aureus*, the cefoxitin disc

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method has better sensitivity than the oxacillin disc method for detecting *mecA*-mediated oxacillin resistance [6]. However, the performance of the cefoxitin and oxacillin disc methods has not been compared in any previous studies that focused on *S. lugdunensis* [7]. In studies that evaluated the prediction of oxacillin resistance in staphylococci in general, few *mecA*-positive *S. lugdunensis* isolates were included [8,9]. In the European Committee on Antimicrobial Susceptibility Testing (EUCAST)'s database of zone diameter distribution, two of 1006 *S. lugdunensis* isolates are resistant by the cefoxitin disc method [10]. Furthermore, recent studies have shown that the test methods (MIC or disc) and breakpoints require modification for some staphylococcal species [11,12]. In the 2019 update of the CLSI M100 document [5], the cefoxitin disc but not oxacillin disc test was considered to be an acceptable method for *S. aureus* and *S. lugdunensis*. On the other hand, the oxacillin disc but not cefoxitin disc was considered to be reliable for *Staphylococcus pseudointermedius* (*S. pseudointermedius*) and *Staphylococcus schleiferi*. Both cefoxitin and oxacillin disc tests were considered to be acceptable for *Staphylococcus epidermidis* (*S. epidermidis*) [5]. In an evaluation of the disc methods for detecting oxacillin resistance in various staphylococcal species, a *mecA*-positive *S. lugdunensis* yielded false-negative results in the cefoxitin disc test (zone diameter 30 mm) [8].

The disc diffusion (DD) test is widely used to detect oxacillin resistance in staphylococci. Due to workflow reasons, many laboratories routinely test both cefoxitin and oxacillin discs. The present study was performed to evaluate disc tests (cefoxitin and oxacillin) and several agar screening methods for detecting *mecA*-mediated oxacillin resistance in *S. lugdunensis*. Oxacillin-resistant staphylococci are considered to be resistant to all β-lactam antibiotics, with the exception of ceftaroline [5].

2. Materials and methods

2.1. Bacterial isolates and identification

A total of 179 *S. lugdunensis* isolates from several published collections, blood culture archives of three regional hospitals and one extended-care hospital in Hong Kong, and archives of carriage studies in medical students were included [1,4,13] (Table S1). Each isolate originated from a different individual. Isolates from patients with healthcare risk factors – including hospital onset (recovery after 2 days of hospitalisation), prior hospitalisation within 6 months, residence in old age home, and long-term renal dialysis – were categorised as healthcare-associated. Isolates from outpatients or inpatients within 2 days of hospitalisation and in which healthcare risk factors were absent were categorised as community-associated. The isolates were stored at −80 °C in MicroBank storage tubes (Pro Lab Diagnostics Inc., TX, USA) and were sub-cultured twice on 5% blood agar before testing. All the isolates were retested in the present study. Identification was achieved by MALDI-TOF and confirmation obtained by a multiplex PCR assay targeting the species-specific thermonuclease (*nuc*) genes [1,4,14].

2.2. Molecular studies

A multiplex PCR was used to detect the *mecA* and *mecC* genes [15]. SCC*mec* types were determined by PCR assays [16]. Multilocus sequence typing (MLST) was performed on strains that were *mecA*-positive [17]. The Institut Pasteur MLST database (https://bigsdb.pasteur.fr/staphlugdunensis/) and the eBURST program were used to assign ST and clonal complex (CC) [17].

2.3. Detection of *mecA*-positive isolates by disc diffusion test, MIC testing and screening with chromID MRSA plates and oxacillin agars

The DD tests (oxacillin and cefoxitin) were performed as described by the CLSI using Muller-Hinton E agar (bioMérieux, Marcy-l'Étoile, France). Isolates that gave discrepant results with *mecA* PCR were further tested by MIC determination using Sensititre plates (Thermo Scientific, UK) containing cation-adjusted Muller-Hinton broth with cefoxitin (unsupplemented cefoxitin 4 μg/mL and 8 μg/mL) and oxacillin (supplemented with 2% NaCl, oxacillin 0.25 μg/mL, 0.5 μg/mL, 1 μg/mL, 2 μg/mL, and 4 μg/mL). Additionally, oxacillin and cefoxitin DD tests were repeated using Muller-Hinton II agar (Becton Dickinson, Hong Kong). In the DD and MIC testing, *Staphylococcus aureus* (*S. aureus*) ATCC 25923 and ATCC 29213 were included for quality control.

The ability of methicillin-resistant *S. aureus* (MRSA) chromogenic and oxacillin agar screening for detecting *mecA*-mediated resistance in *S. lugdunensis* was assessed following the CLSI's procedure [5]. Commercial chromID MRSA plates (bioMérieux, Hong Kong) and in-house Muller-Hinton II (Beckton Dickinson, Hong Kong) agar containing 2% NaCl and 1 μg/mL or 2 μg/mL oxacillin were tested. Bacteria were cultured on 5% horse blood agar plates overnight. At least five colonies were sampled and a 0.5 McFarland standard bacterial suspension was prepared by the colony suspension method. A disposable loop was dipped into the suspension and each agar was spot inoculated an area 10–15 mm in diameter. An inoculum of 1 μL was used for chromID MRSA agar, and 1 μL and 5 μL were used for oxacillin agars. The inoculated plates were incubated at 35 °C in ambient air and examined after 24 h. Absence and presence of growth (>1 colony or thin film) was intercepted as indicating susceptibility and resistance, respectively [5].

2.4. PBP2a test

The penicillin-binding protein 2a (PBP2a) was detected using the MRSA-Screen test (Denka Seiken Co., Ltd., Japan). Colonies grown on 5% blood agar for 24 h were tested according to the manufacturer's instructions and controls (*S. aureus* ATCC 43300 and ATCC 25923) included in each run.

2.5. Data analysis

Disc results were interpreted using the following breakpoints: (a) 2019 CLSI M100 *S. aureus*/*S. lugdunensis* (SA/SL) cefoxitin (resistant, ≤21 mm); (b) 2019 CLSI M100 *S. epidermidis*/other coagulase-negative staphylococcus spp. (SE/CoNS) cefoxitin (resistant, ≤24 mm); (c) 2019 CLSI M100 *S. epidermidis*/*S. pseudointermedius*/*S. schieiferi* (SE/SP/SS) oxacillin (resistant, ≤17 mm); and (d) 2019 EUCAST *S. pseudointermedius* (SP) oxacillin (resistant, ≤19 mm) [5,10]. The *mecA* PCR result was used as the 'gold standard' and compared against the susceptibility test or agar screen results. Categorical agreement (CA), major error (ME), and very major error (VME) were calculated as previously described [11,12]. ME was defined by isolates phenotypically resistant but *mecA*-negative. VME was defined by isolates phenotypically susceptible but *mecA*-positive. The ME and VME rates were calculated by using the total number of *mecA*-negative isolates and *mecA*-positive isolates as denominators, respectively.

3. Results

3.1. Isolates and identification

One hundred seventy-nine isolates were included in this study. Eighty-four isolates were obtained from patients hospitalised for

clinical infections (62 wound infections, 18 bacteraemia, two septic arthritis, one continuous ambulatory peritoneal dialysis peritonitis, and one pneumonia). Ninety-five isolates were collected from patients on long-term renal dialysis (n = 32, 2013–2014) and medical students (n = 63, 2014–2018). The number of isolates with healthcare-associated and community-associated origin was 61 and 118, respectively. All isolates were identified as *S. lugdunensis* by MALDI-TOF and the species confirmed by PCR assays.

3.2. Characteristics of the mecA *positive isolates*

Fifty-two (30 community-associated and 22 healthcare-associated) of the 179 isolates were *mecA* positive. All isolates were *mecC*-negative. The MLST/SCC*mec* types of the *mecA* positive isolates were as follows: ST3/SCC*mec* IV (n = 2), ST3/SCC*mec* V (n = 29), ST27/SCC*mec* V (n = 20), and ST42/SCC*mec* V (n = 1). ST27 and ST42 were assigned as members of CC27.

3.3. Phenotypic susceptibility test agreement with mecA *PCR results*

In the DD tests, there was no clear division of cefoxitin and oxacillin zone diameters between the *mecA*-positive and *mecA*-negative isolates (Fig. 1). For cefoxitin, the CLSI SA/SL breakpoint and CLSI SE/CoNS breakpoint correctly categorised 31 and 34 of the 52 *mecA*-positive isolates, respectively, as resistant (Fig. 1A). For oxacillin, substantial overlaps in the zone diameters for the *mecA*-

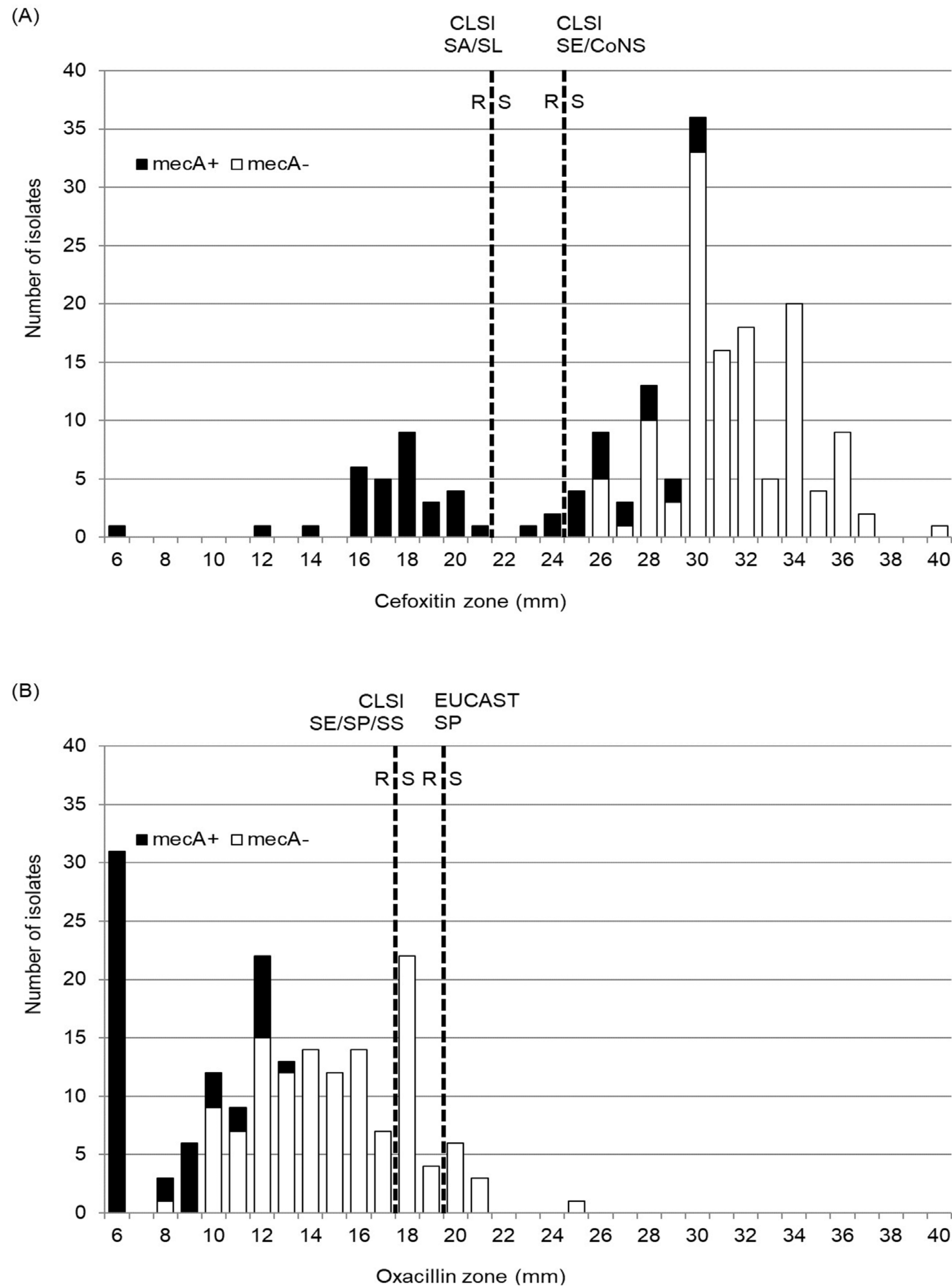

Fig. 1. Distribution of inhibition zone diameters of 179 *Staphylococcus lugdunensis* isolates for (A) cefoxitin and (B) oxacillin.
Cefoxitin breakpoints for *S. aureus*/*S. lugdunensis* (SA/SL) and *S. epidermidis*/other coagulase-negative staphylococci (SE/CoNS); and oxacillin breakpoints for *S. epidermidis*/*S. pseudointermedius* and *S. schleiferi* (SE/SP/SS) according to the Clinical Laboratory Standards Institute (CLSI) M100-2019 and oxacillin breakpoints according to the *S. pseudointermedius* (SP) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) are indicated.

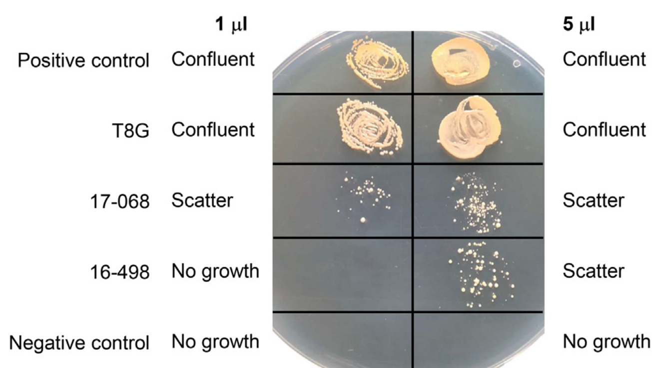

Fig. 2. Growth patterns of representative *Staphylococcus lugdunensis* strains and controls on Muller-Hinton agar supplemented with 2% sodium chloride and 2 μg/mL oxacillin using bacterial inoculum of 1 μL (left panel) and 5 μL inoculum (right panel): *S. lugdunensis* strains of ST3/SCC*mec* V (T8G) and ST27/SCC*mec* V types (17-068, 16-498), showing confluent and scatter growth pattern, respectively; positive control, *Staphylococcus aureus* ATCC 43300; negative control, *S. aureus* ATCC 29213.

positive (range, 6–13 mm) and *mecA*-negative (range, 8–25 mm) isolates were observed (Fig. 1B). Although all *mecA*-positive isolates had zone diameters ≤13 mm, 44 of the 127 *mecA*-negative isolates were incorrectly interpreted as resistant at this cut-off (Fig. 1B).

On applying the CLSI SA/SL and CLSI SE/CoNS breakpoints for cefoxitin DD results, CA was 88% and 90%, respectively; and VME was 40% and 35%, respectively (Table S2). For oxacillin DD, very low CA (35–49%) and high ME (72–86%) were observed upon applying the CLSI SE/SP/SS and EUCAST SP breakpoints. Screening with a bacterial inoculum of 5 μL inoculum using 2 μg/mL oxacillin agars showed 100% CA for all 179 isolates with no ME or VME. In contrast, screening with 1 μL inoculum using oxacillin 2 μg/mL agars, and with 1 μL and 5 μL inoculum using 1 μg/mL oxacillin agars yielded CA values ranging 74–88% (Table S2). Screening with ChromID MRSA agar failed to detect 19 *mecA*-positive isolates, yielding a CA of 89%.

On applying the CLSI SA/SL breakpoint to the cefoxitin DD results, all of the ST3/SCC*mec* IV (n = 2) and ST3/SCC*mec* V (n = 29) isolates were correctly categorised as resistant. In contrast, all the CC27/SCC*mec* V (n = 21) were incorrectly categorised as susceptible.

In the oxacillin agar screen, two different growth patterns were observed. Isolates in the cefoxitin DD-resistant/*mecA*-positive (n = 31) and cefoxitin DD-susceptible/*mecA*-positive (n = 21) groups all yielded confluent and scatter growth patterns, respectively (Fig. 2). The 21 cefoxitin DD-susceptible/*mecA*-positive isolates included 18 isolates from individuals with asymptomatic carriage (16 from medical students and two from patients on long-term renal dialysis) and three from patients with infections (two wound infections and one bacteraemia) (Table 1). The 21 cefoxitin DD-susceptible/*mecA*-positive isolates had cefoxitin and oxacillin MIC ≤ 4 μg/mL and 1–4 μg/mL, respectively. All except one strain were interpreted as oxacillin-susceptible by the MIC tests. Cefoxitin and oxacillin zone diameters for the two brands (bioMérieux and Becton Dickinson) of Muller-Hinton agars were highly consistent (Table 1). The mean (± standard deviation) zone diameters of cefoxitin for bioMérieux Muller-Hinton agar and Becton Dickinson Muller-Hinton agar were 27 ± 2 mm and 26 ± 1 mm, respectively. In the PBP2a test, all 21 isolates showed positive results.

4. Discussion

The data show that cefoxitin DD testing does not accurately predict the presence of *mecA* in *S. lugdunensis* by using the CLSI's M100-2019 zone diameter breakpoint for *S. aureus*/*S. lugdunensis*. This could not be improved by applying cefoxitin breakpoints recommended for other *Staphylococcal* spp. or adding oxacillin DD testing. Cefoxitin DD testing failed to detect oxacillin resistance in 21 of 52 *mecA*-positive isolates. Previous studies have indicated that DD testing of some staphylococci may be confounded by poor

Table 1
Summary of 21 *mecA* positive interpreted as cefoxitin-susceptible by the CLSI M100, 29th edition *S. aureus*/*S. lugdunensis* breakpoint.

Strain	Date collected	Source	Diagnosis	MLST/SCC*mec*	Zone diameter, mm[b,c]		MIC, μg/mL[c]		PBP2a
					Cefoxitin	Oxacillin	Cefoxitin	Oxacillin	
14-031	28 Jan 14	MS	Carriage	ST27/SCC*mec*V	25/26 (S/S)	8/12	≤4 (S)	2 (S)	+
14-025	18 Feb 14	MS	Carriage	ST27/SCC*mec*V	27/26 (S/S)	12/11	≤4 (S)	2 (S)	+
14-009	25 Feb 14	MS	Carriage	ST27/SCC*mec*V	30/29 (S/S)	12/13	≤4 (S)	1 (S)	+
15-170	5 Feb 15	MS	Carriage	ST27/SCC*mec*V	25/26 (S/S)	12/13	≤4 (S)	2 (S)	+
16-498	2 Nov 16	MS	Carriage	ST27/SCC*mec*V	24/27 (S/S)	10/11	≤4 (S)	2 (S)	+
17-037	20 Oct 17	MS	Carriage	ST27/SCC*mec*V	30/27 (S/S)	11/11	≤4 (S)	2 (S)	+
17-065	24 Oct 17	MS	Carriage	ST27/SCC*mec*V	26/26 (S/S)	10/12	≤4 (S)	1 (S)	+
17-068	24 Oct 17	MS	Carriage	ST27/SCC*mec*V	25/26 (S/S)	8/10	≤4 (S)	2 (S)	+
17-151	31 Oct 17	MS	Carriage	ST27/SCC*mec*V	29/27 (S/S)	9/12	≤4 (S)	2 (S)	+
17-115	31 Oct 17	MS	Carriage	ST27/SCC*mec*V	25/28 (S/S)	10/13	≤4 (S)	2 (S)	+
17-126	31 Oct 17	MS	Carriage	ST27/SCC*mec*V	29/26 (S/S)	12/12	≤4 (S)	1 (S)	+
17-174	6 Nov 17	MS	Carriage	ST27/SCC*mec*V	27/26 (S/S)	11/12	≤4 (S)	2 (S)	+
18-111	6 Nov 18	MS	Carriage	ST27/SCC*mec*V	28/25 (S/S)	13/10	≤4 (S)	2 (S)	+
18-128	6 Nov 18	MS	Carriage	ST27/SCC*mec*V	28/27 (S/S)	12/11	≤4 (S)	2 (S)	+
18-166	12 Nov 18	MS	Carriage	ST27/SCC*mec*V	26/26 (S/S)	12/12	≤4 (S)	4 (R)	+
18-191	12 Nov 18	MS	Carriage	ST27/SCC*mec*V	30/28 (S/S)	12/13	≤4 (S)	1 (S)	+
K44G	27 Jan 14	Hosp B	Carriage[a]	ST27/SCC*mec*V	26/27 (S/S)	9/12	≤4 (S)	2 (S)	+
K79G	7 Feb 14	Hosp B	Carriage[a]	ST27/SCC*mec*V	28/26 (S/S)	9/13	≤4 (S)	2 (S)	+
2897	29 Jan 09	Hosp B	Bacteraemia	ST42/SCC*mec*V	26/26 (S/S)	9/10	≤4 (S)	1 (S)	+
9144	17 May 12	Hosp A1	SSTI	ST27/SCC*mec*V	24/25 (S/S)	9/11	≤4 (S)	2 (S)	+
3258	27 Dec 14	Hosp A1	SSTI	ST27/SCC*mec*V	23/22 (S/S)	9/10	≤4 (S)	1 (S)	+

Abbreviations: Hosp, hospital; MLST, multilocus sequence typing; MS, medical student; ST, sequence type; S, susceptible; R, resistant; PBP2a, penicillin-binding protein 2a; +, positive.

[a] Two patients on long-term renal dialysis.

[b] Disc diffusion results obtained using bioMérieux and Becton-Dickinson Muller-Hinton agars were given before and after /, respectively.

[c] The results were interpreted using the CLSI M100-2019 *S. aureus*/*S. lugdunensis* MIC (cefoxitin, S ≤ 4 μg/mL, R ≥ 8 μg/mL; oxacillin S ≤ 2 μg/mL, R ≥ 4 μg/mL) and disc breakpoints (cefoxitin zone diameter, S ≥ 22 mm, R, ≤21 mm).

growth in Muller-Hinton agar medium [11]. The 21 cefoxitin DD-susceptible/*mecA*-positive isolates yielded good growth in the two brands of Muller-Hinton agar media. In the PBP2a test, colonies were harvested from 5% blood agar and positive results could be obtained for the 21 isolates. There was no need to use colonies from the edge of the cefoxitin zone. This was interpreted to indicate an intact *mecA* gene and functional expression in the absence of induction.

All except one of the 21 cefoxitin DD-susceptible/*mecA*-positive isolates had an oxacillin MIC of 1–2 μg/mL and were incorrectly interpreted as oxacillin-susceptible according to the CLSI [5]. For staphylococcal species other than *S. aureus* and *S. lugdunensis*, the CLSI recommend a resistant breakpoint of $\geq$0.5 μg/mL for oxacillin. Previous studies have revealed that many *mecA*-negative *S. lugdunensis* isolates have an oxacillin MIC of 0.5 μg/mL [18]. The current study therefore used oxacillin at 1 μg/mL and 2 μg/mL in agar screening and other oxacillin concentrations were not used. The results showed that 100% CA could be obtained by using 2 μg/mL oxacillin agar screening. At 1 μg/mL oxacillin, a large number of false-positive results were observed for the *mecA* negative isolates (Table S2).

In the oxacillin agar testing, two patterns of growth among the *mecA*-positive isolates were observed. In staphylococci, the production of PBP2a has been reported to result in different patterns of β-lactam resistance [19]. Homogenous oxacillin resistance is a phenotype exhibited by a strain whose entire cell population is uniformly highly resistant to β-lactam antibiotics [19]. The confluent growth obtained for the ST3/SCC*mec* IV or V strains in the oxacillin agar testing indicates that the strains have this resistance phenotype. In the case of heterogeneous resistance, the production of PBP2a yields a strain with a cell population mixed with different levels of β-lactam resistance [19]. The scatter growth pattern exhibited by the CC27/SCC*mec* V in the oxacillin agar testing indicated that they have heterogeneous resistance to oxacillin (Fig. 2). This may provide an explanation as to why a bacterial inoculum of 1 μL is less sensitive than 5 μL in the 2 μg/mL oxacillin agar testing.

The two SCC*mec* types in the current isolates have different combinations of *mec* gene complex (class B in SCC*mec* IV and class C2 in SCC*mec* V) and *ccr* gene complex (*ccrA2B2* in SCC*mec* IV and *ccrC* in SCC*mec* V) [19,20]. The *mec* gene complex comprises the *mec* gene, its regulatory components (*mecR1* and *mecI*) and the associated insertion sequences. In both class B and class C2 *mec* gene complexes, the *mecR1* is truncated and *mecI* is deleted [19,20]. Furthermore, two different oxacillin resistance phenotypes were observed in the ST3/SCC*mec* V and CC27/SCC*mec* V strains. Therefore, the heterogeneous resistance phenotype to oxacillin in the CC27/SCC*mec* V strains is unlikely to be caused by *mecA* regulatory components in the SCC*mec* elements. In experimental studies, the introduction of the *mecA* gene into *S. aureus* produced a heterogeneous pattern of β-lactam resistance [19,21]. Additional chromosomal mutations are required for heterogeneous-to-homogeneous conversion of β-lactam resistance [19,21]. Mutations in the genes encoding the SOS response regulators (*lexA*/*recA*), global regulators (*sar* and *agr*), anti-sigma factor (*rsbW*), and penicillinase *blaZ* regulator (*blaI* and *blaR1*) have been reported to promote heterogeneous-to-homogeneous conversion of oxacillin resistance (or vice versa) in m*ecA*-positive *S. aureus* strains [19,22,23]. It is suggested that mutations required for heterogeneous-to-homogeneous conversion of oxacillin resistance are lacking in the CC27/SCC*mec* V strain. This hypothesis could be further investigated by population analysis profile studies and whole genome sequencing in future studies.

The MLST analysis revealed that the isolates that yielded false-negative results in cefoxitin DD testing were of the CC27/SCC*mec* V clone. Because the bacterial isolates from patients were not consecutively collected in the participating hospitals, no inference can be made on the prevalence of this clone in clinical specimens. Overall, nasal carriage of 18 ORSL (16 CC27/SCC*mec* V and two ST3/SCC*mec* V) were detected from 1267 medical students (Table S1). While the prevalence of carriage of the CC27/SCC*mec* V clone was low (1.3%, 16 of 1267) among the medical students, it was the predominant clone among the ORSL isolates in this cohort. In previous studies, the frequencies of CC27 among *S. lugdunensis* collections were variable [2,24,25]. Yeh et al. reported one of 129 isolates from a regional hospital in 2003–2014 to be ST27 (oxacillin-sensitive) [2]. Dahyot et al. analysed 127 isolates from hospitals in multiple French regions in 2013–2016 and reported two to be ST27 (oxacillin susceptibility not reported) [24]. By contrast, 10 of 40 *S. lugdunensis* from rectal swabs at a Japanese hospital between 2002 and 2008 were typed as ST27; all 10 ST27 isolates were oxacillin-susceptible [25].

This study was limited by the inclusion of isolates from a small geographic area and only a subset of the isolates was examined by MIC testing. Nonetheless, the strengths of this study included the enrolment of isolates from multiple sources over different time periods and a relatively large number of *mecA*-positive isolates being evaluated.

In conclusion, this study showed that oxacillin resistance in a *mecA*-positive *S. lugdunensis* clonal complex 27 clone cannot be reliably detected by the current disc (cefoxitin) and MIC (cefoxitin and oxacillin) breakpoints. These findings highlight the need for additional tests, such as screening with 2 μg/mL oxacillin agar using a high bacterial inoculum or PCR assays, to accurately detect these strains. Future studies to systematically assess *S. lugdunensis* isolates from different geographical areas by a combination of PCR assays and phenotypic susceptibility tests are required to help clinicians choose more appropriate antimicrobial therapy for patients.

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Competing interests

None.

Ethical approval

Not required.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2019.08.021.

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