Infection and Drug Resistance

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Dissemination Of t437-SCC*mec*IV And Coagulase-Negative t037-SCC*mec*III Types Among Borderline Oxacillin-Resistant *Staphylococcus aureus* Isolated From Skin Infections And Diabetic Foot Ulcers

This article was published in the following Dove Press journal:
Infection and Drug Resistance

Maria Stańkowska[1]
Katarzyna Garbacz[1]
Lidia Piechowicz[2]
Marek Bronk[3]

[1]Department of Oral Microbiology, Faculty of Medicine, Medical University of Gdansk, Gdansk, Poland; [2]Department of Medical Microbiology, Faculty of Medicine, Medical University of Gdansk, Gdansk, Poland; [3]Laboratory of Clinical Microbiology, University Clinical Center, Gdansk, Poland

Background: In a recent decade, the occurrence of *S. aureus* isolates with low-level oxacillin resistance, have been reported increasingly. The aim of this study was to estimate the prevalence of *S. aureus* with low-level of oxacillin resistance and to determine their molecular characteristics, including *spa* types, SCC*mec* types and presence of toxin genes.

Methods: A total of 249 *S. aureus* strains were analyzed. Antimicrobial susceptibility was preliminarily tested by the disk diffusion method, and further was verified with the E-test and agar dilution methods. All borderline oxacillin-resistant strains (BORSA) were screened for the *mec*A gene and virulence factors, including Panton-Valentine leukocidin (PVL). Staphylococcal cassette chromosome *mec* (SCC*mec*) typing and *spa* typing were also carried out.

Results: Twelve (4.8%) borderline oxacillin-resistant strains with MIC ≤4 µg/mL were identified. Almost all strains (11/12) were oxacillin-susceptible methicillin resistant *S. aureus* carrying *mecA* gene (OS-MRSA). Among the 12 bordeline strains, five *spa* types (t437, t037, t015, t216, t267) and two SCC*mec* types (III, IV) were identified, with the most prevalent being t437-SCC*mec*IV *pvl*-positive. The second most frequent *spa* type, t037-SCC*mec*III, was *sea*-positive and did not produce coagulase. The majority of borderline strains originated from skin infections and diabetic foot ulcers and were multidrug-resistant (macrolides, lincosamides and chloramphenicol).

Conclusion: This study demonstrated that *S. aureus* with borderline resistance to oxacillin represented primarily SCC*mec*IV *spa* type t437 and coagulase-negative SCC*mec*III *spa* type t037 and were isolated from skin infections and diabetic foot ulcers.

Keywords: *Staphylococcus aureus*, MRSA, OS-MRSA, borderline oxacillin-resistant *S. aureus*, low-level oxacillin resistance

Introduction

Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) constitute a global public health burden. Minimum inhibitory concentration (MIC) of oxacillin for most MRSA strains exceeds 4 µg/mL; oxacillin-resistance is determined by the presence of the *mecA* gene encoding penicillin-binding protein 2a (PBP2a). However, in recent years, clinical MRSA strains with low-level resistance to oxacillin were isolated in several countries including the Netherlands, Germany, Greece, Taiwan, China, Japan, the USA and Angola.[1–6] The strains with low-level resistance to oxacillin are also referred to as borderline oxacillin-resistant *S. aureus* (BORSA) or oxacillin-

Correspondence: Katarzyna Garbacz
Department of Oral Microbiology, Medical University of Gdansk, 25 Dębowa St, Gdansk 80-204, Poland
Tel +48 58 349 1900
Fax +48 58 349 1668
Email katarzyna.garbacz@gumed.edu.pl

submit your manuscript | www.dovepress.com
DovePress
http://doi.org/10.2147/IDR.S219557

susceptible MRSA (OS-MRSA). This group includes two subsets of strains, *mec*A-positive and *mec*A-negative. The mechanism of phenotypic resistance to oxacillin in the *mec*A-negative strains that harbor neither *mec*A nor *mec*C gene is still not fully understood. Probably resistance of these strains to oxacillin is associated with overproduction of beta-lactamases or, in some cases, with point mutations in PBP genes.[7,8] Borderline isolates may be easily misidentified as methicillin-sensitive *S. aureus* (MSSA), which may result in treatment failure and spread of these strains within the hospital and community setting.[9] Epidemiology and clinical presentation of infections caused by BORSA seem to be similar to those associated with MRSA; usually, the infections are more severe than those caused by MSSA, even if a larger dose of oxacillin is administered.[10] According to Huang et al,[11] bacteremia caused by BORSA was significantly more often associated with pneumonia or empyema than the systemic infections caused by MSSA and MRSA. Available evidence suggests that the problem of BORSA/OS-MRSA spread is frequently underestimated, and considering their documented emergence in new environments, these strains need to be monitored and accurately distinguished from MSSA.[12]

The aim of this study was to estimate the prevalence of borderline oxacillin-resistant *S. aureus* and to determine their molecular characteristics, including *spa* types, SCC*mec* types and presence of toxin genes.

Materials And Methods

Bacterial Strains

A total of 249 non-duplicate *S. aureus* methicillin-resistant strains from various clinical samples were analyzed. The study was based on a retrospective analysis of methicillin-resistant strains isolated within the last 21 years and archived at the Department of Medical Microbiology, and the strains from the Laboratory of Clinical Microbiology, University Clinical Center in Gdansk, isolated during routine clinical laboratory procedures. The identity of *S. aureus* isolates was verified with conventional methods and with Pastorex Staph-Plus latex agglutination kit (Bio-Rad, Marnes-la-Coquette, France) and confirmed based on the polymerase chain reaction (PCR) of *S. aureus*-specific region of the thermonuclease gene, *nuc*.[13] The isolates were stored at −80°C in trypticase soy broth (TSB) (Oxoid, Basingstoke, England) supplemented with 15% glycerol.

Antimicrobial Susceptibility

The antimicrobial susceptibility of *S. aureus* strains was tested by disk diffusion method using penicillin G, erythromycin, clindamycin, ciprofloxacin, tetracycline, gentamicin, sulfamethoxazole/trimethoprim, chloramphenicol and fusidic acid discs from Becton Dickinson (Franklin Lakes, NJ, USA) and was interpreted according to the Clinical and Laboratory Standards Institute (CLSI).[14] MIC for vancomycin was determined by E-tests, in line with the manufacturer's instructions (AB Biodisc, Solna, Sweden).

Detection Of Methicillin Resistance

All archival strains that were previously labeled as methicillin-resistant were subjected to repeated analysis. Susceptibility to oxacillin and cefoxitin was preliminarily determined by the disk diffusion method on Mueller-Hinton agar (Becton Dickinson, Franklin Lakes, NJ, USA). The plates were incubated for 24 h at 35°C, and the results were interpreted according to the CLSI.[14] In the case of the strains with discordant results of oxacillin and cefoxitin susceptibility tests, oxacillin MIC was additionally determined with the agar dilution test, in line with the CLSI guidelines,[15] and by means of E-test (Becton Dickinson, Franklin Lakes, NJ, USA). In brief, selected *S. aureus* strains were plated on Mueller-Hinton agar supplemented with 4% sodium chloride containing 1, 2, 4, and 6 μg/mL oxacillin. The results were recorded after 24-h and 48-h incubation at 35°C. The strains with oxacillin MICs ≤4 μg/mL on agar dilution test were considered BORSA. All selected strains were tested for the presence of *mecA* gene using the method described previously elsewhere.[16] Moreover, one *mec*A-negative strain was tested for the carriage of *mec*C gene.[17] The reference strains, *S. aureus* ATCC 43300 and *S. aureus* ATCC 29213, were used as positive and negative controls, respectively.

Coagulase Production

For all borderline oxacillin-resistant strains free coagulase test was performed under standard conditions by incubating 0.8 mL of TSB culture with 0.2 mL rabbit plasma for 2, 4, 6 and 24 h at 37°C.[18] The reference strains, *S. aureus* ATCC 29213 and *S. epidermidis* PCM 2118, were used as positive and negative controls, respectively.

Molecular Typing

Total DNA of *S. aureus* isolates was purified using Genomic DNA kit (A&A Biotechnology, Gdynia, Poland), in line with the manufacturer's instructions.

Typing of the staphylococcal chromosomal cassette *mec* (SCC*mec*) was carried out as described previously by Milheiriço et al.[16] Genes encoding enterotoxins (*sea, seb, sec, sed*), exfoliative toxins (*eta, etb*), toxic shock syndrome toxin-1 (*tst*) and Panton-Valentine leukocidin (*lukS*-PV/*lukF*-PV) were detected by means of multiplex PCR, as described elsewhere.[19,20] The presence of penicillinase encoded by *bla Z* gene was determined according to Wang et al.[21] The PCR products were electrophoretically resolved in 1.5% agarose containing 0.5 µg/mL ethidium bromide.

spa Typing

spa typing was performed according to Harmsen et al.[22] Nucleotide sequencing of the repeat-containing region of the *spa* gene was conducted on both DNA strands of the PCR product from Genomed (Warszawa, Poland), using BigDye Terminator Ready Reaction Cycle Sequencing kit (St Louis, MO, United States). *spa*-types were identified with Ridom Staph-Type software v.2.1.1 (Ridom GmbH, Münster, Germany).[22]

Statistical Analysis

Distributions of quantitative variables were presented as numbers and percentages and compared between groups using the Pearson chi-square or Fisher exact test. All calculations were carried out with Statistica 10 package (StatSoft, Tulsa, OK, United States), with the threshold of statistical significance set at $p \leq 0.05$.

Results

Prevalence Of Borderline Oxacillin-Resistant *S. aureus*

The analyzed group of 249 *S. aureus* included 12 (4.8%) identified as borderline oxacillin-resistant strains based on oxacillin MIC ≤4 µg/mL determined with the agar dilution method. The strains were isolated from wounds (n=5), foot ulcers (n=3), furuncle (n=1), catheter (n=1), blood (n=1) and nose (n=1) (Table 1).

Antimicrobial Susceptibility

MICs of oxacillin for the 12 bordeline strains varied between 2 µg/mL and 4 µg/mL when determined with agar dilution method and between 1 µg/mL and 4 µg/mL if estimated using the E-test. 9 out of 12 BORSA strains were susceptible to oxacillin but resistant to cefoxitin, whereas 3 out of 12 isolates were susceptible to cefoxitin but resistant to oxacillin. Moreover, BORSA strains turned out to be resistant to erythromycin (11/12), clindamycin (7/12), chloramphenicol (6/12), tetracycline (4/12), ciprofloxacin (3/12), doxycycline (3/12) and gentamicin (3/12). Most BORSA (10/12) were identified as multidrug-resistant strains, and aside from beta-lactams showed also resistance to other antibiotics (Table 1).

Molecular Characteristics

Five *spa* types were identified among the analyzed borderline strains. Half (6/12) of them represented type t437, one-fourth

Table 1 Characteristics Of Borderline Oxacillin-Resistant *S. aureus*

Patient No.	*spa* Type	Year	Origin	MIC Of Oxacillin (µg/mL)		Resistance To (DD, mm)		Phenotype Of Resistance	Coagulase Production
				AD	E-Test	OX	FOX		
1	t037	1998	Wound	4	2	12 R	25 S	OX, E, CC, DX, GE, CIP, C	–
2	t037	2000	Foot ulcer	2	1	12 R	26 S	OX, E, CC, DX, GE	–
3	t037	2000	Wound	2	1	11 R	23 S	OX, E, DX, GE	–
4	t437	2013	Furuncle	4	2	14 S	19 R	FOX, E, CC, TE, C	+
5	t437	2013	Wound	4	2	15 S	19 R	FOX, E, CC, TE, C	+
6	t437	2013	Wound	4	2	14 S	19 R	FOX, E, CC, TE, C	+
7	t437	2016	Foot ulcer	2	1	15 S	18 R	FOX, E, CC, TE, C	+
8	t437	2017	Wound	4	2	13 S	17 R	FOX	+
9	t437	2018	Foot ulcer	4	1.5	17 S	19 R	FOX, E, CC, C	+
10	t216	2013	Nose	4	1.5	14 S	16 R	FOX, E, CIP	+
11	t267	2016	Blood	4	4	15 S	19 R	FOX, E	+
12	t015	2018	Catheter	4	2	18 S	18 R	FOX, E, CIP	+

Abbreviations: AD, agar dilution; DD, disk diffusion; OX, oxacillin; FOX, cefoxitin; E, erythromycin; CC, clindamycin; DX, doxycycline; TE, tetracycline; GE, gentamycin; CIP, ciprofloxacin; C, chloramphenicol; R, resistant; S, sensitive.

(3/12) belonged to type t037, and single strains were identified as types t015, t216 and t267. Almost all strains (11/12) carried *mecA* and *blaZ* genes. The strains carried SCC*mec* type IV (9/12) or SCC*mec* type III (2/12). The list of identified toxin genes included *lukF-lukS-PV* (5/12), *seb* (5/12), *sea* (3/12) and *tst* (1/12) (Table 2, Figure 1).

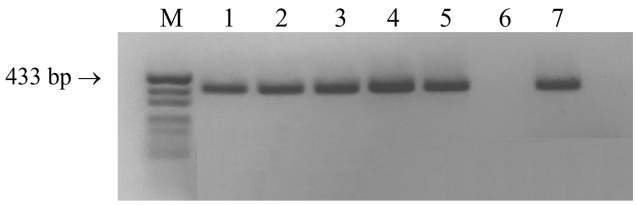

Figure 1 Agarose gel image of *lukS-PV/lukF-PV* PCR amplicon (433 bp). Lane M – molecular size marker (*pUC19 DNA/MspI enzyme*, Fermentas, Lithuania); lanes 1-5 – representative PVL-positive *S. aureus* strains of *spa* type t437, lane 6 – negative control; lane 7 – positive control.

Comparison Between *spa* Types t437 And t037

Borderline strains belonging to type t437 were identified twice as often as those from type t037 and were isolated from more recently archived clinical material. Strains belonging to both *spa* types originated from skin infections and diabetic foot ulcers. Type t437 and t037 differed in terms of their molecular characteristics; while the strains of type t437 carried toxin genes *pvl* (5/6), *seb* (4/6), and SCC*mec* type IV, those representing type t037 harbored *sea* (3/3) and *tst* (1/3) genes and SCC*mec* type III. Most of the t437 strains were resistant to clindamycin (5/6), erythromycin (5/6) and chloramphenicol (5/6), whereas all isolates belonging to the *spa* type t037 showed a distinct pattern of resistance to doxycycline and gentamycin (3/3). Unlike the t437 strains, none of the t037 isolates produced coagulase (Table 3).

Discussion

In a recent decade, the occurrence of *S. aureus* isolates with low-level oxacillin resistance, especially *mecA*-positive strains, have been reported increasingly. Published data about the prevalence of these isolates differ considerably depending on the country and clinical material, from slightly above 1% (1.1% in Taiwan, 1.25% in Japan, 1.6% in China),[3,4,23] to a few percent (5.8–9.8%),[2,24] or even more than 10% or 20%.[5,25] Considering these data, the prevalence of borderline strains in our material should be considered moderately high.

According to literature, borderline *S. aureus* are isolated from skin and soft tissue infections, bacteremia, pneumonia and urinary tract infections.[11] The majority of these strains identified in our study originated from skin infections; to the best of our knowledge, our study was the first to document the presence of borderline resistant strains in diabetic foot ulcers. In recently published paper, Lin et al[26] did not specify whether the t437 strains isolated from diabetic foot ulcers showed low-level oxacillin resistance or were fully resistant to this antibiotic.

Most previously isolated OS-MRSA were identified as community-acquired MRSA (CA-MRSA) strains. Based on

Table 2 The Presence Of Virulence And Resistance Genes To β–Lactams Antibiotics In Different *spa* And SCC*mec* Types Of Borderline Oxacillin-Resistant *S. aureus*

Patient No.	*spa* Type	Repeat Succession	SCC*mec* Type	Genetic Profile										
				mecA	*mecC*	*blaZ*	*lukF-lukS-PV*	*sea*	*seb*	*sec*	*sed*	*tst*	*eta*	*etb*
1	t037	15-12-16-02-25-17-24	III	+	–	+	–	+	–	–	–	–	–	–
2	t037	15-12-16-02-25-17-24	–	–	–	+	–	+	–	–	–	+	–	–
3	t037	15-12-16-02-25-17-24	III	+	–	+	–	+	–	–	–	–	–	–
4	t437	04-20-17-20-17-25-34	IV	+	–	+	+	–	–	–	–	–	–	–
5	t437	04-20-17-20-17-25-34	IV	+	–	+	+	–	+	–	–	–	–	–
6	t437	04-20-17-20-17-25-34	IV	+	–	+	+	–	+	–	–	–	–	–
7	t437	04-20-17-20-17-25-34	IV	+	–	+	–	–	–	–	–	–	–	–
8	t437	04-20-17-20-17-25-34	IV	+	–	+	+	–	+	–	–	–	–	–
9	t437	04-20-17-20-17-25-34	IV	+	–	+	+	–	+	–	–	–	–	–
10	t216	04-20-17-20-17-31-16-34	IV	+	–	+	–	–	+	–	–	–	–	–
11	t267	07-23-12-21-17-34-34-34-33-34	IV	+	–	+	–	–	–	–	–	–	–	–
12	t015	08-16-02-16-34-13-17-34-16-34	IV	+	–	+	–	–	–	–	–	–	–	–

Table 3 Characteristics Of Borderline Oxacillin-Resistant *S. aureus* *spa* Types t037 And t437

Parameter	*spa* Type t037	*spa* Type t437	p-Value
Year of isolation	1998–2000	2013–2018	
Prevalence	3/12	6/12	0.400
Skin infection	2/3	4/6	1.000
Foot ulcer	1/3	2/6	1.000
SCC*mec*III	3/3	0/6	**0.012**
SCC*mec*IV	0/3	6/6	**0.012**
sea	3/3	0/6	**0.012**
seb	0/3	4/6	0.167
lukF-lukS-PV	0/3	5/6	**0.048**
tst	1/3	0/6	0.333
Clindamycin/erythromycin resistance	2/3	5/6	1.000
Gentamicin/doxycycline resistance	3/3	0/6	**0.012**
Production of coagulase	0/3	6/6	**0.012**

Note: Bold values indicate statistical significance.

their molecular characteristics, also the strains identified in our study were probably CA-MRSA. Similar to many previous reports from Asia, most of our OS-MRSA strains represented *spa* type t437 classified among the international CA-MRSA clones.[24] This implies that the type t437 staphylococci have already expanded onto the European continent. CA-MRSA strains typically harbor staphylococcal cassette chromosome *mec* type IV (SCC*mec*IV), along with the *pvl* gene which is considered a genetic marker for this group.[27] Nearly half of the strains identified in our study were SCC*mec*IV-*pvl*-positive, which is consistent with the reports from other European centers,[6] and distinguishes these clones from those isolated in Asia, more often being SCC*mec*V-*pvl*-negative.[11] Presence of Panton-Valentine toxin was shown to be associated with furunculosis and necrotizing pneumonia, but as emphasized by Gillet et al,[28] this relationship was observed primarily in healthy children and young adults.

To the best of our knowledge, our study was the first to identify coagulase-negative *S. aureus* isolates belonging to *spa* type t037 and showing borderline resistance to oxacillin. Previously, type t037 strains were found among both MRSA and OS-MRSA,[24,26,29] but none of the reported isolates was coagulase-negative. Skinner et al[30] reported on a patient in whom endocarditis was caused by a strain that showed borderline resistance to oxacillin and lacked either thermonuclease or coagulase, i.e. the two basic taxonomic characteristics of *S. aureus*. Aside from difficulties in the selection of appropriate antibiotic therapy, another challenge in that case was the identification of the isolate at a species level.[30] Missing expression of species-specific proteins has been reported primarily in the case of MRSA; this phenomenon may result from the insertion of a transposon (Tn917) or integration of plasmid genes into the chromosome.[12] According to Duval-Iflah et al,[31] the loss of ability to coagulate plasma may result from a lysogenic conversion with LS1 and LS2 phages.

Borderline *S. aureus* isolates are usually multidrug-resistant; this was also confirmed in our present study in which most of the strains showed resistance to macrolides and lincosamides, but unlike the isolates from Asia, were relatively often susceptible to fluoroquinolones.[11] Treatment of infections caused by OS-MRSA may be challenging; according to Ho et al,[3] nearly half of patients infected with these strains received inadequate treatment due to misidentification of the etiological factor as MSSA, which in two cases had fatal consequences.

It is still unclear which epidemiological and/or clinical factors contributed to the spread of the *S. aureus* with low-level resistance to oxacillin across all continents. One may stipulate if a likely cause of BORSA spread is their better adjustment to environmental conditions with lesser antibiotic selective pressure than in the case of typical MRSA and whether these strains can be even more widespread in future.[32] Recent findings suggest that the MRSA clones might simultaneously co-evolve in different geographic regions.

Conclusion

This study demonstrated that *S. aureus* with borderline resistance to oxacillin represented primarily SCC*mec*IV *spa* type t437 and coagulase-negative SCC*mec*III *spa* type t037 and were isolated from skin infections and diabetic foot ulcers. Future studies should monitor the spread of these strains and related risks associated with their involvement in other infections.

Abbreviations

BORSA, borderline oxacillin-resistant *S. aureus* (BORSA); CA-MRSA, community-acquired methicillin-resistant *S. aureus*; CLSI, Clinical and Laboratory Standards Institute; MIC, minimum inhibitory concentration (MIC); MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; OS-MRSA, oxacillin-susceptible *S. aureus*; PBP, penicillin-binding protein; PCR, polymerase chain reaction; *S. aureus, Staphylococcus aureus*; SCC*mec*, staphylococcal cassette chromosome *mec*; TSB, trypticase soy broth.

Acknowledgments

The study was supported by a specific subsidy of Ministry of Science and Higher Education for Medical University of Gdansk grant, no. 01-0328/08/402 and ST02-0543/07/289. The Authors would like to express their gratitude to Dr. Szymon Bruzewicz for this linguistic assistance in the preparation of the manuscript.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Conceição T, Coelho C, de Lencastre H, Aires-de-Sousa M. Frequent occurrence of oxacillin-susceptible mecA-positive *Staphylococcus aureus* (OS-MRSA) strains in two African countries. *J Antimicrob Chemother*. 2015;70(12):3200–3204. doi:10.1093/jac/dkv261
2. Forbes BA, Bombicino K, Plata K, et al. Unusual form of oxacillin resistance in methicillin-resistant *Staphylococcus aureus* clinical strains. *Diagn Microbiol Infect Dis*. 2008;61(4):387–395. doi:10.1016/j.diagmicrobio.2008.04.003
3. Ho C-M, Lin C-Y, Ho M-W, et al. Methicillin-resistant *Staphylococcus aureus* isolates with SCCmec type V and spa types t437 or t1081 associated to discordant susceptibility results between oxacillin and cefoxitin, Central Taiwan. *Diagn Microbiol Infect Dis*. 2016;86(4):405–411. doi:10.1016/j.diagmicrobio.2016.08.025
4. Hososaka Y, Hanaki H, Endo H, et al. Characterization of oxacillin-susceptible mecA-positive *Staphylococcus aureus*: a new type of MRSA. *J Infect Chemother*. 2007;13(2):79–86. doi:10.1007/s10156-006-0502-7
5. Petinaki E, Kontos F, Maniatis AN. Emergence of two oxacillin-susceptible mecA-positive *Staphylococcus aureus* clones in a Greek hospital. *J Antimicrob Chemother*. 2002;50(6):1090–1091. doi:10.1093/jac/dkf235
6. Wannet WJ, Spalburg E, Heck ME, Pluister GN, Willems RJ, De Neeling AJ. Widespread dissemination in the Netherlands of the epidemic berlin methicillin-resistant *Staphylococcus aureus* clone with low-level resistance to oxacillin. *J Clin Microbiol*. 2004;42(7):3077–3082. doi:10.1128/JCM.42.7.3077-3082.2004
7. McDougal LK, Thornsberry C. The role of beta-lactamase in staphylococcal resistance to penicillinase-resistant penicillins and cephalosporins. *J Clin Microbiol*. 1986;23(5):832–839.
8. Tomasz A, Drugeon HB, de Lencastre HM, Jabes D, McDougall L, Bille J. New mechanism for methicillin resistance in *Staphylococcus aureus*: clinical isolates that lack the PBP 2a gene and contain normal penicillin-binding proteins with modified penicillin-binding capacity. *Antimicrob Agents Chemother*. 1989;33(11):1869–1874. doi:10.1128/aac.33.11.1869
9. Cuirolo A, Canigia LF, Gardella N, et al. Oxacillin- and cefoxitin-susceptible meticillin-resistant *Staphylococcus aureus* (MRSA). *Int J Antimicrob Agents*. 2011;37(2):178–179. doi:10.1016/j.ijantimicag.2010.10.017
10. Balslev U, Bremmelgaard A, Svejgaard E, Havstreym J, Westh H. An outbreak of borderline oxacillin-resistant *Staphylococcus aureus* (BORSA) in a dermatological unit. *Microb Drug Resist*. 2005;11(1):78–81. doi:10.1089/mdr.2005.11.78
11. Huang YT, Liao CH, Chen SY, Hsu HS, Teng LJ, Hsueh PR. Emergence of multidrug-resistant sequence type 45 strains among mecA-positive borderline oxacillin-resistant *Staphylococcus aureus* causing bacteraemia in a medical centre in Taiwan. *Int J Antimicrob Agents*. 2018;52(1):70–75. doi:10.1016/j.ijantimicag.2018.02.014
12. Hryniewicz MM, Garbacz K. Borderline oxacillin-resistant *Staphylococcus aureus* (BORSA) – a more common problem than expected? *J Med Microbiol*. 2017;66(10):1367–1373. doi:10.1099/jmm.0.000585
13. Brakstad OG, Aasbakk K, Maeland JA. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. *J Clin Microbiol*. 1992;30(7):1654–1660.
14. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. Document M100-S25*. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
15. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. Document M02-A11*. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
16. Milheiriço C, Oliveira DC, de Lencastre H. Update to the multiplex PCR strategy for assignment of mec element types in *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 2007;51(9):3374–3377. doi:10.1128/AAC.00275-07
17. Cuny C, Layer F, Strommenger B, Witte W. Rare occurrence of methicillin-resistant *Staphylococcus aureus* CC130 with a novel mecA homologue in humans in Germany. *PLoS One*. 2011;6(9):e24360. doi:10.1371/journal.pone.0024360
18. Kloos W, Lambe D. Staphylococcus. In: Balows A, Hausler W, editors. *Manual of Clinical Microbiology*. Washington, DC: American Society for Microbiology; 1991:222–237.
19. Becker K, Friedrich AW, Lubritz G, Weilert M, Peters G, Von Eiff C. Prevalence of genes encoding pyrogenic toxin superantigens and exfoliative toxins among strains of *Staphylococcus aureus* isolated from blood and nasal specimens. *J Clin Microbiol*. 2003;41(4):1434–1439. doi:10.1128/jcm.41.4.1434-1439.2003
20. Becker K, Roth R, Peters G. Rapid and specific detection of toxigenic *Staphylococcus aureus*: use of two multiplex PCR enzyme immunoassays for amplification and hybridization of staphylococcal enterotoxin genes, exfoliative toxin genes, and toxic shock syndrome toxin 1 gene. *J Clin Microbiol*. 1998;36(9):2548–2553.
21. Wang SK, Gilchrist A, Loukitcheva A, et al. Prevalence of a cefazolin inoculum effect associated with *blaZ* gene types among methicillin-susceptible *Staphylococcus aureus* isolates from four major medical centers in Chicago. *Antimicrob Agents Chemother*. 2018;62(8). doi:10.1128/AAC.00382-18.
22. Harmsen D, Claus H, Witte W, et al. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. *J Clin Microbiol*. 2003;41(12):5442–5448. doi:10.1128/jcm.41.12.5442-5448.2003
23. Song Y, Cui L, Lv Y, Li Y, Xue F. Characterisation of clinical isolates of oxacillin-susceptible mecA-positive *Staphylococcus aureus* in China from 2009 to 2014. *J Glob Antimicrob Resist*. 2017;11:1–3. doi:10.1016/j.jgar.2017.05.009
24. Chen FJ, Hiramatsu K, Huang IW, Wang CH, Lauderdale TL. Panton-Valentine leukocidin (PVL)-positive methicillin-susceptible and resistant *Staphylococcus aureus* in Taiwan: identification of oxacillin-susceptible mecA-positive methicillin-resistant *S. aureus*. *Diagn Microbiol Infect Dis*. 2009;65(4):351–357. doi:10.1016/j.diagmicrobio.2009.07.024
25. Chen FJ, Huang IW, Wang CH, et al. mecA-positive *Staphylococcus aureus* with low-level oxacillin MIC in Taiwan. *J Clin Microbiol*. 2012;50(5):1679–1683. doi:10.1128/JCM.06711-11
26. Lin SY, Lin NY, Huang YY, Hsieh CC, Huang YC. Methicillin-resistant *Staphylococcus aureus* nasal carriage and infection among patients with diabetic foot ulcer. *J Microbiol Immunol Infect*. 2018. doi:10.1016/j.jmii.2018.03.005

27. Vandenesch F, Naimi T, Enright MC, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. *Emerg Infect Dis*. 2003;9(8):978–984. doi:10.3201/eid0908.030089
28. Gillet Y, Issartel B, Vanhems P, et al. Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. *Lancet*. 2002;359(9308):753–759. doi:10.1016/S0140-6736(02)07877-7
29. Asadollahi P, Farahani NN, Mirzaii M, et al. Distribution of the most prevalent *spa* types among clinical isolates of methicillin-resistant and -susceptible *Staphylococcus aureus* around the world: a review. *Front Microbiol*. 2018;9:163. doi:10.3389/fmicb.2018.00163
30. Skinner S, Murray M, Walus T, Karlowsky JA. Failure of cloxacillin in treatment of a patient with borderline oxacillin-resistant *Staphylococcus aureus* endocarditis. *J Clin Microbiol*. 2009;47(3):859–861. doi:10.1128/JCM.00571-08
31. Duval-Iflah Y, Van Heijenoort J, Rousseau M, Raibaud P. Lysogenic conversion for multiple characters in a strain of *Staphylococcus aureus*. *J Bacteriol*. 1977;130(3):1281–1291.
32. Pournaras S, Stathopoulos C, Tsakris A. Oxacillin-susceptible MRSA: could it become a successful MRSA type? *Future Microbiol*. 2013;8(11):1365–1367. doi:10.2217/fmb.13.118