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Case Report

Fatal sepsis caused by *mecA*-positive oxacillin-susceptible *Staphylococcus aureus*: First report in a tertiary hospital of southern Brazil☆

Felipe Crepaldi Duarte [a], Tiago Danelli [a], Eliandro Reis Tavares [b, c], Ana Elisa Belotto Morguette [c], Gilselena Kerbauy [d], Cintia Magalhães Carvalho Grion [e], Lucy Megumi Yamauchi [c, f], Marcia Regina Eches Perugini [a, g, **], Sueli Fumie Yamada-Ogatta [a, c, f, *]

[a] *Programa de Mestrado em Fisiopatologia Clínica e Laboratorial, Departamento de Patologia, Análises Clínicas e Toxicológicas, Centro de Ciências da Saúde, Universidade Estadual de Londrina, Brazil*
[b] *Bolsista do Programa Nacional de Pós-Doutorado do Programa de Pós-graduação em Microbiologia, Departamento de Microbiologia, Centro de Ciências Biológicas, Universidade Estadual de Londrina, Brazil*
[c] *Laboratório de Biologia Molecular de Microrganismos, Departamento de Microbiologia, Centro de Ciências Biológicas, Universidade Estadual de Londrina, Brazil*
[d] *Departamento de Enfermagem, Centro de Ciências da Saúde, Universidade Estadual de Londrina, Brazil*
[e] *Departamento de Clínica Médica, Centro de Ciências da Saúde, Universidade Estadual de Londrina, Brazil*
[f] *Programa de Pós-graduação em Microbiologia, Departamento de Microbiologia, Centro de Ciências Biológicas, Universidade Estadual de Londrina, Brazil*
[g] *Laboratório de Microbiologia Clínica, Departamento de Patologia, Análises Clínicas e Toxicológicas, Centro de Ciências da Saúde, Universidade Estadual de Londrina, Brazil*

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ABSTRACT

mecA-positive oxacillin phenotypically susceptible *Staphylococcus aureus* (OS-MRSA) is increasingly reported worldwide. This bacterium poses a therapeutic threat, as it can be misidentified as an oxacillin-susceptible organism by phenotypic methods that are routinely used in the majority of clinical microbiology laboratories. Herein, we report the first case of fatal sepsis in a 43-year-old female patient caused by an OS-MRSA SCC*mec* type IVa/ST1/CC1 in a tertiary hospital in southern Brazil, which highlights the difficulties involved in diagnosing this bacterium. Blood cultures and phenotypic susceptibility tests on admission yielded a penicillin-resistant *S. aureus*. Although vancomycin therapy was initiated, this antibacterial was replaced by oxacillin, based on the susceptibility result. However, the clinical conditions of the patient deteriorated rapidly evolving to fatal septic shock. Clinical microbiology laboratories should consider the use of additional tests to accurately distinguish between various antimicrobial phenotypes of *S. aureus*.

1. Introduction

Staphylococcus aureus is a leading cause of bacteremia worldwide, often associated with poor outcomes. Besides the clinical course, which can lead to complications such as infective endocarditis, sepsis and septic shock, the antimicrobial resistance poses important challenges in the treatment of this infection [1,2]. Currently, a high proportion of bacteremia is caused by methicillin-resistant *Staphylococcus aureus* (MRSA), which besides resistance to β-lactams, has become resistant to nearly all antibacterial agents

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* Corresponding author. Departamento de Microbiologia, Centro de Ciências Biológicas, Universidade Estadual de Londrina, Rodovia Celso Garcia Cid, PR445, km 380, Campus Universitário, Londrina, CEP 86057-970, Paraná, Brazil.
** Corresponding author. Hospital Universitário de Londrina, Departamento de Patologia, Análises Clínicas e Toxicológicas, Avenida Robert Koch, 60, Vila Operária, Londrina, CEP 86038-350, Paraná, Brazil.
E-mail addresses: marciaperugini@uel.br (M.R.E. Perugini), ogatta@uel.br (S.F. Yamada-Ogatta).

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used to treat it, limiting therapeutic options [1]. The most common mechanism of methicillin-resistance is mediated by the acquisition of *mec* genes (*mecA*, *mecB* or *mecC*) that encode the penicillin-binding protein 2a (PBP2a) with low affinity to β-lactam antibacterials [3,4].

Resistance to *in vitro* cefoxitin-disk screen test or an oxacillin minimum inhibitory concentration (MIC) of ≥4 μg/mL are routinely used for phenotypic identification of MRSA in clinical laboratories [5]. Moreover, the presence of *mec* genes, generally detected by nucleic acid amplification tests, has been used as genetic marker of MRSA [3]. However, isolates carrying *mecA* gene but phenotypically susceptible to cefoxitin/oxacillin, referred to as oxacillin-susceptible MRSA (OS-MRSA), have been reported worldwide [6–13]. These isolates are prone to be misidentified as methicillin-susceptible *S. aureus* (MSSA), leading to therapeutic failure. Herein, we present a fatal case of infection caused by an OS-MRSA in a tertiary hospital in southern Brazil.

2. Case report

A 43-year-old Caucasian female was referred from a local hospital to the emergency room of our hospital with a seven day history of edema and pain in lower members. Significant medical history included type-2 diabetes with irregular use of metformin, recurrent furunculosis and urinary tract infections. On admission, she presented with a low level of consciousness, abdominal pain, and was using intravenous ceftriaxone (2 g/12 h). Her vital signs examination revealed a Systemic Inflammatory Response Syndrome [14] (heart rate: 95 beats/min; temperature: 37.5 °C; respiratory rate: 22 breaths/min; leukocytes count: 24,800/mm^3), score quick Sequential Organ Failure Assessment (qSOFA) = 3, (respiratory rate: 22 breaths/min; altered mentation; systolic blood pressure: 72 mmHg) and score SOFA = 16 [15]. Respiratory dysfunction was detected, requiring intubation with mechanical ventilator support and she was sedated. Sepsis diagnosis was confirmed. Initial laboratory examination (on day 1) showed moderate anemia (hematocrit: 28.4 g/dL; hemoglobin: 11.0 g/dL; platelet count: 153 × 10^9/L). High levels of C-reactive protein (502.7 mg/L; reference value < 9 mg/L) and lactate (7.3 mmol/L) were detected. Furthermore, she presented hypotension requiring vasopressor drug therapy despite adequate fluid resuscitation. Septic shock diagnosis was confirmed [15] and ceftriaxone was replaced empirically by piperacillin-tazobactam (2.25 g/6 h) initiated 24 hours following admission. Blood cultures were performed, 2 hours following the patient admission, using the BD BACTEC™ Automated System (Becton–Dickinson, USA) and yielded Gram-positive *cocci* reported presumptively as *Staphylococcus* spp. Thus, on day 2, intravenous vancomycin (initial dose of 1 g, followed by 500 mg/6 h) was added to the antimicrobial therapy, and clinical improvements were observed. The use of vasopressor drug was discontinued. On day 3, species identification and antibiotic susceptibility profile generated by Vitek2® System AST-P585 card (bioMérieux, USA) revealed MSSA resistant only to penicillin [5]. Hence, vancomycin therapy was discontinued and intravenous oxacillin (2 g/4 h) was initiated. Five days after the initial oxacillin treatment, the patient did not present any improvement in her clinical condition. Serial blood cultures and antimicrobial susceptibility testing again identified MSSA resistant to penicillin. On the 6th day of oxacillin therapy, the patient went into septic shock and vasopressor drug was reintroduced into therapy. Oxacillin was discontinued and daptomycin (500 mg/24 h) was introduced; however, the patient died 10 days after admission. A time line summarizing the clinical and laboratorial examinations and the therapy are described in Table 1.

Further analysis confirmed the identification of all isolates (named as 549, 550 and 551) as *S. aureus* by multiplex-PCR [16]. The antimicrobial susceptibility profile of the isolates was reported in Table 2. A heteroresistant population was not observed, even in isolates (550 and 551) recovered after oxacillin therapy or *in vitro* induction with subinhibitory concentration of the antimicrobial, as determined by the population analysis profile (PAP) [17] generated after 24 and 48 hours-incubation. The *mecA* gene was detected and according to the multiplex-PCR described by Milheiriço et al. [18], this gene was inserted into the staphylococcal cassette chromosome (SCC)*mec* type IVa. The High Resolution Melting (HRM) analysis of Single Nucleotide Polymorphisms (SNP) [19] revealed that the isolates belonged to the Sequence Type (ST) 1 and Clonal Complex (CC) 1. Study protocols were approved by Ethics Committee of Universidade Estadual de Londrina (CAAE no.78657317.0.0000.5231-CEP-UEL).

3. Discussion

Herein, we described a case of fatal sepsis caused by an OS-MRSA SCC*mec* type IVa/ST1/CC1 in a female patient admitted to a tertiary hospital of southern Brazil, which was misidentified as MSSA by phenotypic methods. Methicillin-resistance among *Staphylococcus* species can be detected by: 1) oxacillin MIC for *S. aureus* and coagulase-negative *staphylococci*; 2) cefoxitin MIC for *S. aureus* and *S. lugdunensis*; 3) 30 μg cefoxitin disk-diffusion assay for *S. aureus* and coagulase-negative *staphylococci*; and 4) 6 μg/mL oxacillin and 4% NaCl agar screening test for *S. aureus*. Moreover, if the isolate tests positive for the *mecA* gene, it should be reported as oxacillin-resistant [5]. However, discrepant results among phenotypic methods, and *mecA* gene or PBP2a detection for MRSA identification have been reported [6–13].

The term OS-MRSA for the description of *S. aureus* harboring the *mecA* gene but susceptible to oxacillin was first used in Japan by Hososaka et al. [8]. These authors identified six (1.25%) OS-MRSAs, among 480 *S. aureus* clinical isolates, exhibiting MIC values (as determined by the agar dilution assay) ranging from 0.5 to 2.0 μg/mL. These isolates were positive for both β-lactamase production by nitrocefin disk method and the presence of *blaZ* gene. Isolates matching the OS-MRSA features had been reported previously by Sakoulas et al. [6] in the USA and Kampf et al. [7] in Germany. In the first study [6], a prevalence of 0.62% of OS-MRSA (2 out of 324 clinical isolates) was observed and this phenotype was detected by the oxacillin susceptibility profile generated by Vitek-1 GPS-106 and Vitek-2 AST-GP 55 cards, agar dilution and oxacillin screen test (Muller Hinton supplemented with 4% NaCl and 6 μg/mL oxacillin). Kampf et al. [7] reported a prevalence of 4.65% of OS-MRSA (7 out of 151 isolates from nasal and oropharyngeal carriage surveillance swabs) by using 1 μg oxacillin disk-diffusion and Iso-Sensitest broth supplemented with 2% NaCl and 2 μg/mL oxacillin assays.

Currently, the occurrence of OS-MRSA has been reported in different countries in the world, showing a prevalence rate of 0.62–33.7% [6–8,10,11,13]. In Brazil, Andrade-Figueiredo and Leal-Balbino [10] reported the highest prevalence of OS-MRSA (33.7%, 30 out of 89 *S. aureus* clinical isolates) in hospitals in Recife in northeast Brazil. In these hospitals, methicillin resistance was phenotypically identified by using the cefoxitin disk-diffusion or agar dilution assays, which failed to identify OS-MRSA. The presence of *mecA* gene was detected by PCR. These authors observed a clonal spread of OS-MRSA within several areas of the investigated hospitals, which may explain the high incidence of this phenotype. Further studies should be conducted to identify an overall prevalence of OS-MRSA in Brazilian hospitals.

A limitation of our report is that we did not perform a systematic analysis of all bloodstream infections over the period of this study.

Table 1
Timing of the clinical and laboratory features and therapy for the patient: the first case of OS-MRSA in a tertiary hospital of southern Brazil.

Variable	Illness day (Date in 2016)									
	1 (12 July)	2 (13 July)	3 (14 July)	4 (15 July)	5 (16 July)	6 (17 July)	7 (18 July)	8 (19 July)	9 (20 July)	10 (21 July)
Clinical feature										
Heart rate (beats/min)	94–128	90–151	106–88	93–120	82–110	76–113	106–131	110–135	123–159	151–154
Respiratory rate (breaths/min)	28–30	28–36	29–32	32–35	30–35	26–30	17–33	16–30	11–37	38
Temperature (°C)	37.1–39.8	36.3–38.4	37.0–38.4	35.9–39.0	36.1–38-6	35.5–37.3	37.3–38.0	36.4–39.0	38.0–41.3	40.0
Systolic Blood Pressure (mmHg)	63–90	71–90	75–92	63–108	60–90	66–87	71–90	65–90	58–107	46–77
Laboratory examination										
Leukocyte count (mm^3)	13,770–24,800	13,070–13,730	14,040	10,080	–	10,480	14,210	–	–	17,710
C-reactive protein (mg/dL)[a]	410.7–502.7	338.8–366.4	347.7	224.9	178.0	236.4	221.5	179.1	237.6	–
Lactate (mmol/L)[b]	2.6–7.3	2.1–2.3	2.7–2.8	2.7	2.5	–	–	2.3	2.9–6.4	–
Oxygen saturation (%)	86.0	99.0	95.0–99.0	98.0	97.0	–	–	93.0	97.0	91.0
Creatinine (mg/dL)	3.24	4.36–4.72	3.64	3.76	4.27–4.50	–	4.26	4.80	4.75	4.50
Blood culture[c]	*S. aureus* 549	–	–	–	–	–	*S. aureus* 550	*S. aureus* 551	–	–
Therapy										
Antimicrobial (dose/hour)	Ceftriaxone (2 g/12 h)	Piperaracillin-tazobactam (2.25 g/6 h)[d] Vancomycin (initial dose of 1 g, and after 500 mg/6 h)[e]	Oxacillin (2 g/4 h)[f]	Oxacillin (2 g/4 h)	Oxacilin (2 g/4 h)	Oxacillin (2 g/4 h)	Oxacillin (2 g/4 h)	Oxacilin (2 g/4 h)	Daptomicyn (500 mg/24 h)[g]	Daptomicyn (500 mg/24 h)
Life Support	Vasopressor drug Mechanical ventilation	– Mechanical ventilation Hemodialysis	– Mechanical ventilation Hemodialysis	– Mechanical ventilation Hemodialysis	– Mechanical ventilation Hemodialysis	– Mechanical ventilation Hemodialysis	– Mechanical ventilation Hemodialysis	Vasopressor drug Mechanical ventilation Hemodialysis	Vasopressor drug Mechanical ventilation Hemodialysis	Vasopressor drug Mechanical ventilation

–: Indicates no results in the day or no vasopressor drug in use.
[a] Reference value < 9 mg/L.
[b] Reference value < 1.1 mg/dL.
[c] Initial blood culture was performed 2 h following admission.
[d] Empirical treatment due to septic shock diagnosis.
[e] Empirical treatment due to the presumptive identification of Gram positive *cocci* in blood culture.
[f] Methicillin susceptible *S. aureus* diagnosis was confirmed and vancomycin was replaced by oxacillin.
[g] Patient went into septic shock and oxacillin was replaced by daptomycin. Data of the Clinical feature and Laboratory Examination were presented as the lower and higher results detected during the day of the analyses.

Table 2
Antimicrobial susceptibility profile and clonal type of *mecA*-positive oxacillin suceptible *Staphylococcus aureus* (OS-MRSA) isolated from blood culture[a] of inpatient of tertiary hospital in southern Brazil.

Variable	MIC[b]	Category
Vitek2®AST-P585 card		
Ciprofloxacin	≤0.5	S
Clindamicyn	≤0.25	S
Erythromycin	≤0.25	S
Gentamicin	≤0.5	S
Penicillin	≥0.5	R
Rifampicin	≤0.5	S
Tigecycline	≤0.12	S
Trimethoprim/sulfamethoxazole	≤10.0	S
***E*test®**		
Daptomycin	0.38	S
Linezolid	4.0	S
Oxacillin	0.75	S
Vancomycin	1.0	S
Disk-diffusion[c]		
Cefoxitin disk (30 μg)	30 mm	S
Genotyping		
SCC*mec* type[d]		IVa
Clonal complex[e]		CC1
Sequence type[e]		ST1

Vitek2 card (bioMérieux, USA).
E-test (AB Biodisk, Sweden).
[a] Blood cultures were performed on illness days 1, 7 and 8, and all isolates exhibited the same results.
[b] Values were expressed in μg/mL, except when specified.
[c] CLSI [5].
[d] Multiplex-PCR assay according to Miheiriço et al. [18].
[e] HRM-SNP analysis according to Lilliebridge et al. [19].

The results would provide better insight into the prevalence of OS-MRSA in our hospital. Despite this limitation, the finding of OS-MRSA poses an additional threat in the antimicrobial therapy of staphylococcal infections. The use of β-lactams agents could select for highly resistant bacterial cells within the population, leading to treatment failure. Indeed, Jones et al. [20] showed that failure in antimicrobial therapy was significantly higher in patients with bloodstream infections caused by OS-MRSA compared to those caused by MRSA.

The choice and timing of antimicrobial therapy greatly affect the outcome of severe bacteremia [1,21]. The first blood culture performed in our institution detected Gram-positive *cocci* in the first 18 hours of incubation, after which broad-spectrum vancomycin therapy was empirically administered according to the hospital guidelines. Since all bloodstream cultures yielded bacteria presenting characteristics compatible with the phenotypic identification of MSSA, vancomycin de-escalation was performed and oxacillin therapy was initiated.

In vitro studies have shown that OS-MRSA can consist of a very heteroresistant population, where the majority of cells are susceptible to low concentrations of oxacillin/methicillin, but a small proportion of the cells are able to grow at higher concentrations of the antimicrobial [6,7,11,12]. Furthermore, the resistant subpopulation has often displayed high resistance level, showing increased MIC values on exposure to oxacillin [7,12,22], cefotaxime [7] or mupirocin [22]. The study of Prouxl et al. [12] is noteworthy as it reported the *in vivo* reversion of methicillin susceptibility to methicillin resistance in *mecA*-positive MSSA in a patient during antimicrobial therapy. The authors also showed that the reversion can occur *in vitro* at a frequency of approximately 10^{-7}, and oxacillin significantly increased the rates of this event. In contrast, a heteroresistant population in OS-MRSA isolates was not observed even after *in vitro* and *in vivo* exposure to oxacillin in this study. Similarly, the growth in the presence of subinhibitory concentrations of cefoxitin did not change the MIC values of this antimicrobial for OS-MRSA [10].

The mechanism by which some MRSA isolates display susceptibility to oxacillin is still obscure, however some evidence has contributed to a better understanding of the nature of this phenotype: 1) the transposable element IS*1181* inserted into the *mecA* gene prevents the expression of PBP2a, and is precisely excised in the presence of oxacillin, restoring the resistant phenotype [12]; 2) deletion of a single nucleotide into the region coding for the N-terminal of PBP2a, a region with unknown function, through slipped-strand mispairing, yields a frameshift mutation and an early stop codon in the *mecA* gene of the susceptible strain, and the insertion of one nucleotide in the same region reverts to the resistant phenotype [12]; 3) mutation into the *femXAB* genes that encode proteins involved in the pentaglycine bridge biosynthesis, can affect the cell wall formation, but not the susceptibility to oxacillin [11,23]; 4) a plasmid-encoded non-functional N-terminally truncated *blaR1* sensor-transducer gene leads to repression of the *mecA* gene, even in the presence of β-lactams [24]. In view of these data, now, we are faced with the task of trying to understand the mechanism of OS-MRSA isolated in this study.

In the present study, the identification of *S. aureus* phenotypically susceptible to oxacillin may have favored the negative outcome of the patient, since it led to inadequate therapy. Although there is still no data on the prevalence of OS-MRSA phenotype in our institution, our findings highlight the need for additional tests to accurately distinguish between various antimicrobial susceptibility phenotypes of *S. aureus*, which are not routinely performed by clinical microbiology laboratories in many countries, including in Brazil. Data from these assays would help clinicians to choose more appropriate antimicrobial therapy for patients.

Authors' contribution

F.C.D.: Contributed to all methodological activities and analysis and interpretation of data; T.D., E.R.T. and A.E.B.M.: Performed the microbiological experiments and analyzed the data; L.M.Y. and C.M.C.G.: Interpretation of data and critical revision of the manuscript for important intellectual content. G.K., S.F.Y.-O. and M.R.E.P.: Conception, design, analysis and interpretation of data. All authors read and approved the final manuscript.

Conflicts of interest

None.

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