

# Methicillin-resistant *Staphylococcus lugdunensis* carrying SCCmec type V misidentified as MRSA

## ABSTRACT

*Staphylococcus lugdunensis* is a rare cause of severe infections and clinical manifestations are similar to those related to *S. aureus* infection. We describe a hospital-acquired bacteremia due to methicillin-resistant *Staphylococcus lugdunensis*, misidentified as methicillin-resistant *S. aureus*. The oxacillin MIC was 16 µg/mL and the *mecA* gene and SCCmec type V were determined by PCR. Although treatment had been appropriated, the patient died after rapid progressive respiratory failure and another nosocomial sepsis. It is important not only to identify *S. lugdunensis* in view of its clinical course, but also to determine its susceptibility to oxacillin by detecting the *mecA* gene or its product.

**Keywords:** *Staphylococcus*; methicillin resistance; bacteremia.

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## CASE REPORT

A 73-year old female with systemic arterial hypertension and chronic obstructive pulmonary disease (COPD) GOLD IV was admitted to the Emergency Department of the University Hospital 'Clementino Fraga Filho' (HUCFF), in March 2005. HUCFF, a tertiary-care teaching hospital with 490 beds, is affiliated to the Federal University of Rio de Janeiro, Rio de Janeiro City, Brazil. The patient had asthenia, cough, fever, purulent secretions and worsening of dyspnea, but the chest x-ray showed no pulmonary infiltrates. Diagnosis of acute exacerbation and upper respiratory infection were made. Bronchodilators, glucocorticoids, gatifloxacin and non-invasive ventilation were initiated. Respiration did not recover, and mechanical ventilation was needed. After completing the course of antimicrobial therapy the patient recovered. However, two cultures from blood collected at day 7 of therapy detected Gram-positive bacteria. Bacterial contamination of blood was initially suspected since the patient had improved and precise identification of the sites of blood collection was not possible. Initially, methicillin-resistant *S. aureus* (MRSA) and *Streptococcus* alpha

hemolytic were identified in the hospital laboratory three days after blood collection and the strains were sent to our laboratory for confirmation. After the initial clinical improvement, the patient presented fever, leucocytosis and hypotension. The central line catheter was removed, and vancomycin and amikacin were included in the treatment, according to the local microbiology pattern and the previous isolation. The nosocomial sepsis was probably related to the central line catheter,<sup>1</sup> although abdominal imaging were not performed. The transthoracic echocardiogram result was normal. Another set of blood cultures drawn on the day when sepsis was diagnosed detected *Escherichia coli* and *S. epidermidis*. The patient had fast and progressive respiratory failure and despite intense medical treatment she expired on day 18 of hospitalization.

The isolate obtained from the first blood culture was characterized as MRSA in the hospital laboratory by VITEK (BioMerieux). Gram staining, catalase test and latex agglutination clumping factor and DNase detection were performed as complementary tests. Antimicrobial susceptibility testing (VITEK, BioMerieux) revealed resistance to oxacillin, penicillin and chloramphenicol.

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In our laboratory, the isolate presented slightly yellow colonies and showed beta-hemolysis on sheep blood agar. It gave a positive reaction for clumping factor, but the tube coagulase test was negative. Then, the isolate was evaluated using the method based on the conventional reference tests<sup>2</sup> and the ornithine decarboxylase production, an enzyme produced only by *S. lugdunensis* strains.<sup>3</sup> To confirm the species identification, the *fbl* gene, which encodes a fibrinogen binding protein of *S. lugdunensis* was amplified by PCR as previously published.<sup>2</sup> A 425 bp amplicon was obtained after PCR amplification of template DNA extracted from reference strain *S. lugdunensis* DSMZ 4804 and the clinical isolate. Antimicrobial susceptibility was evaluated according to CLSI guidelines. The isolate was resistant to oxacillin, ceftioxin, penicillin and chloramphenicol by disk diffusion test. Oxacillin MIC, determined by the E-test method, was 16 µg/mL. Initially, the *mecA* gene was detected by PCR, and the SCCmec typing was performed by multiplex PCR of the *ccr* type and *mec* class, using the method described by Kondo *et al.*<sup>4</sup> The isolate was *ccr* type 5 and *mec* class C, characterizing the SCCmec type V. The 804 bp amplicon (corresponding to *ccr5* gene) and 518 bp (corresponding to class C *mec* gene) generated in PCR reaction were confirmed by sequencing and comparison with GenBank database.

This is the first case report of a methicillin-resistant *S. lugdunensis* (MRSL) isolate carrying the SCCmec type V causing a systemic infection. To our knowledge, this is the fourth report in the literature describing *S. lugdunensis* carrying the *mecA* gene. Kawaguchi *et al.*<sup>5</sup> detected the *mecA* gene in one of two strains of *S. lugdunensis* isolated from blood during a laboratory surveillance in Japan, but no description of clinical infection was provided by them. In 2003, Tee *et al.*<sup>6</sup> reported a case of MRSL causing bloodstream infection in a neonate. In 2008, Tan, Ng and He<sup>7</sup> found 4.7% *S. lugdunensis* strains carrying the *mecA* gene in a collection of 106 clinical isolates. In Brazil, this is the first report of a MRSL isolate.

*S. lugdunensis* was first described by Freney<sup>8</sup> and is associated with several infections, mainly skin/soft tissues infections and endocarditis.<sup>9</sup> In the clinical setting it frequently causes illness that has been described as acute, aggressive and destructive - more in keeping with coagulase-positive staphylococci than characteristically indolent coagulase-negative staphylococcal infections, especially endocarditis, where the majority of infections involve native or prosthetic valves, being the mortality rates high.<sup>10</sup> Therefore, its aggressive nature resembles *S. aureus*. *S. lugdunensis* should be suspected when a staphylococcal isolate is positive in the clumping factor test but negative in the tube coagulase test.<sup>6</sup> The isolation rates of *S. lugdunensis* are about 10% for non-*S. epidermidis* coagulase negative isolates.<sup>3</sup> However, in a recent study, Böcher *et al.*<sup>11</sup> showed that the misidentification of *S. lugdunensis* could be

contributing to this low rate. They improved the identification of *S. lugdunensis* using the Columbia sheep-horse agar inoculation as an additional test. To reinforce this fact we evaluated the bacterial diagnostic provided for 5,360 blood cultures performed in our hospital, between 2000 and 2009 (data not shown). No *S. lugdunensis* isolate was detected, suggesting that this pathogen is being misidentified.

In the present case report we observed a clear misidentification of a MRSL isolate as community MRSA. Both species present similar colony morphology and blood-agar hemolysis. Interestingly, in addition to being positive by the clumping factor agglutination test, *S. lugdunensis* strains are also positive for DNase, like *S. aureus* strains. This shows the importance to employ tube coagulase testing for staphylococcal characterization. For confirmation the ornithine-decarboxylase test is also important, since *S. lugdunensis* is the only staphylococcus positive in this assay. The susceptibility pattern of the isolate indicated a community-associated pathogen. Only resistance for β-lactams and chloramphenicol were observed. Moreover, this non-multiresistance has been related to the SCCmec type V, often found in community isolates. This case report confirms the isolation of non-multiresistant *S. lugdunensis* carrying the SCCmec V as a cause of a serious hospital-acquired infection which was misdiagnosed as non-multiresistant MRSA in a setting with nosocomial transmission of the latter.

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