*Rev. Inst. Med. Trop. Sao Paulo*
*54(6):305-306, November-December, 2012*

# COMPARISON OF FIVE METHODS FOR OXACILLIN SUSCEPTIBILITY TESTING OF *Staphylococcus aureus* ISOLATES FROM CYSTIC FIBROSIS PATIENTS

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## SUMMARY

Methicillin-resistant *Staphylococcus aureus* (MRSA) are now a worldwide problem. Cystic fibrosis (CF) patients are commonly colonized and infected by MRSA. Accurate oxacillin susceptibility testing is mandatory for the adequate management of these patients. We performed a comparison of the accuracy of different tests in CF isolates, including methicillin-susceptible *S. aureus* and MRSA with different SCC*mec* types, and using the *mecA* gene as the gold-standard. The sensitivity and specificity of oxacillin disc, Etest, and oxacillin agar screening plate were 100%. Sensitivity of the cefoxitin disc was 85% and specificity was 100%. For clinically relevant isolates, laboratories may consider the use of a combination of two phenotypic methods.

**KEYWORDS:** Oxacillin; Cefoxitin; *Staphylococcus aureus; mecA;* Cystic fibrosis; MRSA.

## INTRODUCTION

During the last decade, new clones of methicillin- resistant *S. aureus* (MRSA) have emerged in different parts of the world, including our country[9]. Part of these novel isolates are community-associated MRSA (CA-MRSA), typically carrying a Staphylococcal Cassette Chromosome *mec* (SCC*mec*) type IV, and also *lukS-PV* and *lukF-PV*, the genes encoding Panton-Valentine leukocidin, a specific virulence factor[10].

Cystic fibrosis patients are frequently colonized and infected with *Staphylococcus aureus*, including MRSA[7]. Accurate detection of methicillin resistance in cystic fibrosis isolates is vital, since even in non-invasive isolates an inaccurate susceptibility test could lead to inappropriate antimicrobial therapy.

Although the *mecA* gene, that encodes methicillin resistance in *S. aureus*, is considered the gold standard test, it is not generally used in routine clinical testing, because of financial and technical issues. There are several studies in the biomedical literature regarding different oxacillin phenotypic susceptibility tests for *S. aureus*[1,2,4-6,8,12-15,17,18], but, to our knowledge, none has included only cystic fibrosis isolates. Thus, the aim of the present study was to determine the accuracy of phenotypic oxacillin susceptibility methods in *Staphylococcus aureus* cystic fibrosis isolates with different SCC*mec* types.

## METHODS

Our pediatric department is part of a large, tertiary general hospital in São Paulo, Brazil. In our cystic fibrosis clinic, 110 patients are followed monthly or bimonthly. In these occasions, upper respiratory tract samples are routinely collected for culture.

We included in our study *S. aureus* isolated from these patients during the period from January 2004 to December 2005. We included only one isolate for patient (the first isolate during the study period).

Isolates were tested with oxacillin (1 µg) and cefoxitin (30 µg) discs, using Mueller-Hinton agar plates inoculated with a suspension (equivalent to a 0.5 McFarland standard) of the *S. aureus* clinical isolates. The plates were incubated at 35 ºC for 24 hours and inhibition zones were measured. The susceptibility to oxacillin was also determined by Etest (AB Biodisk, Solna, Sweden), using Mueller-Hinton agar plates supplemented with 2% NaCl, and by oxacillin agar screening, that was performed by inoculating a direct colony suspension (0.5 McFarland standard) with a swab, spotting an area 10 to 15 mm in diameter, on Mueller-Hinton agar supplemented with 4% NaCl and oxacillin at 6 mg/L. After incubation for 24 hours, any growth was interpreted as a positive result for MRSA.

The inhibition zones and minimum inhibitory concentration breakpoints used for interpretation were those recommended by CLSI[3]. The *mecA* gene was detected by polymerase chain reaction (PCR), as described by VANNUFFEL *et al.*[16], and was considered the gold standard test for oxacillin resistance. SCC*mec* typing was performed with a multiplex (PCR) protocol previously described by OLIVEIRA *et al.*[11]. The study was approved by the Ethics Committee at our institution.

## RESULTS AND DISCUSSION

During the study period, there were 30 *S. aureus* isolates from different patients (27% of the patients were colonized with *S. aureus* at least once during the period). Seven were *mecA*-positive. The specificity of all four phenotypic methods, using the *mecA* results as gold standard, was 100%. The sensitivity of oxacillin disc, Etest, and oxacillin screening

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plate was also 100%. The sensitivity of the cefoxitin disc test was 85%, due to a discrepant result in one isolate.

Of the seven *mecA*-positive isolates, four were available for SCC*mec* typing. Of these four, three carried SCC*mec* type III and one carried SCC*mec* type IV. The isolate with SCC*mec* IV and one of the three *mecA*-positive isolates that were unavailable for typing were resistant only to penicillin, oxacillin and erythromycin. All other five isolates were multiresistant.

Most clinical laboratories throughout the world rely on disc diffusion testing for the detection of methicillin resistance in *S. aureus*. Other available routine tests include epsilometer test and oxacillin agar screening plate. Although recommended by guidelines and validated in multiple studies[1-6,8,12-15,17,18], there is a lack of data about the accuracy of these tests in isolates from such a specific population as cystic fibrosis patients.

In our study, performed in a relatively small number of isolates, we demonstrated the possibility of occurrence of major errors, with a high potential for clinical impact, when only one of this tests is used. Errors in routine oxacillin susceptibility tests, including disc diffusion with either oxacillin or cefoxitin with different breakpoints, have also been reported elsewhere[4,8,14]. Laboratories may consider using two methods (cefoxitin disc plus oxacillin disc; or oxacillin agar plate plus disc diffusion, per example) when testing clinically relevant isolates.

## RESUMO

**Comparação entre cinco métodos para avaliação de susceptibilidade à oxacilina em cepas de *Staphylococcus aureus* isoladas de pacientes com fibrose cística**

*Staphylococcus aureus* resistentes à oxacilina (MRSA) são, atualmente, um problema global. Pacientes com fibrose cística (FC) são frequentemente colonizados e infectados por MRSA. A realização de testes de susceptibilidade acurados é extremamente importante para o manejo da terapia antimicrobiana nesses indivíduos. Nesse estudo, realizamos comparação entre as acurácias de diversos testes de susceptibilidade à oxacilina, em cepas de *S. aureus* isoladas de pacientes com fibrose cística, tanto sensíveis como resistentes à oxacilina, com diferentes tipos de SCC*mec*, e utilizando a detecção do gene *mecA* como método padrão. A sensibilidade e a especificidade do disco de oxacilina, do Etest, e da placa de agar *screening* com oxacilina foram de 100%. A sensibilidade do disco de cefoxitina foi 85%, com especificidade de 100%. Em cepas clinicamente relevantes, a utilização combinada de mais de um método deveria ser considerada.

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Received: 7 May 2012
Accepted: 10 July 2012