Assessment of three rapid methods for the detection of methicillin-resistant Staphylococcus aureus

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We evaluated three rapid methods to detect methicillin-resistant Staphylococcus aureus (MRSA) and compared them with PCR amplification of meca. A total of 103 S. aureus strains were studied by MRSA-Screen, BBL Crystal, Velogene Genomic and meca PCR. All the methods detected the 61 MRSA strains having the meca gene, showing 100% sensitivity and specificity. Despite the correlation between all the rapid methods and PCR, the ease of use and shorter turnaround time of MRSA-Screen were important factors leading to the selection of this method as the routine screening technique for MRSA.

Key words: MRSA, MRSA-Screen, BBL Crystal, Velogene, meca-PCR.

Evaluación de tres métodos rápidos para la detección de Staphylococcus aureus resistente a la meticilina

Se han evaluado tres métodos rápidos para detectar la presencia de Staphylococcus aureus resistente a la meticilina (SARM) y se han comparado con la amplificación del gen meca mediante reacción en cadena de la polimerasa (PCR). Se estudiaron un total de 103 cepas de S. aureus mediante MRSA-Screen, BBL Crystal, Velogene Genomics y PCR para meca. Con todos estos métodos se detectaron 61 cepas de SARM que presentaban el gen meca, con una sensibilidad y especificidad del 100%. A pesar de la correlación entre todos los métodos rápidos y la PCR, la facilidad de uso y el poco tiempo que lleva a la realización de MRSA-Screen fueron factores importantes para la selección de este método como técnica sistemática de detección de SARM.

Palabras clave: SARM, MRSA-Screen, BBL Crystal, Velogene, meca-PCR.

Introduction

Methicillin resistant Staphylococcus aureus (MRSA) is an important pathogen causing severe nosocomial infections whose prevalence has been increasing, both in Europe and USA. Given the clinical and epidemiological importance of MRSA isolates at the hospital setting, the rapid identification of MRSA is of paramount importance. Methicillin resistance in Staphylococcus aureus is due to the hyperproduction of the low-affinity penicillin-binding protein PBP2a, coded by the meca gene whose presence is not detected in methicillin sensitive S. aureus strains.

Although PCR meca gene amplification is considered as the "gold standard" for MRSA detection, several commercial fast methods for the detection of methicillin resistance are now available.

The femA gene, highly conserved among Staphylococci, has been used for the identification of Staphylococci species. In the present study, we evaluated three of such methods for the MRSA detection, and compared them with the PCR detection of the meca gene. The methods evaluated were:

2. BBL crystal MRSA ID System. This test uses an oxygen sensitive fluorophore, which fluoresces in the presence of MRSA derived oxygen consumption.
3. Velogene Genomic ID assay. A qualitative DNA test using an meca derived RNA/DNA chimeric probe labelled both with biotin and fluorescein. The hybridization with meca positive strain DNA releases the RNA portion of the chimeric probe, which after degradation by RNase is no longer reactive with the peroxidase labelled anti-fluorescein antibody. Thus this test results in colour generation only if no MRSA strain is present.

Material and methods

Isolates. A total of 103 S. aureus strains isolated from different clinical samples at Hospital Geral do Santo António were studied. These isolates were identified by the VITEK® system (bioMérieux). In all test batches ATCC25923, a methicillin sensitive S. aureus (MSSA), and a MRSA strain positive for the meca gene were included as controls.

MRSA-Screen (Immunogenetics, Japan), BBL Crystal MRSA ID (Becton Dickinson, USA) and Velogene Genomic ID Assay (Alexis-Trend, Canada). These methods were performed according to the respective manufacturer’s instructions, from subcultures in blood agar.

PCR. PCR amplification of meca gene was used as golden standard method. Briefly, nucleic acid extraction was performed from a 500 μl aliquot of Tryptic Soy broth 18 hour liquid culture. The pellet...
obtained after centrifugation (7,500 rpm; 10 min) was resuspended in 100 µl Tris EDTA buffer (Qiagen, USA) and was lysed with 10 µl of Sigma lysozyme (SIGMA) for one hour at 37 °C. DNA extraction was performed in the Magna-Pure LC® (Roche, Germany) using the Magna-Pure LC Total Nucleic Acid Isolation kit (Roche) and the External Lysis protocol.

A multiplex PCR assay with simultaneous detection of mecA and femA gene was performed as previously described with a few modifications: 25 µM of each primer M1 and M2 (mecA gene), 100 µM of each primer F1 and F2 (femA gene), 2.5 mM MgCl2, 2 IU TaqNAzyme EXT (Finnzymes Oy, Finlandia) in the manufacturer supplied buffer. Amplicons size was characterized by agarose electrophoresis using precast 4% NuSieve® 3:1 Plus Agarose gels (BMA,USA). Amplification of the femA gene works both as a species confirmation signal and as an internal positive control of the PCR reaction.

Results

PCR products showed a 310bp fragment corresponding to the mecA gene in 61 strains, whereas all 103 strains showed a 666bp fragment corresponding to the femA gene (data not shown) confirming the presence of S. aureus.

All methods assayed revealed the presence of 61 MRSA strains corresponding to the mecA gene positive strains by PCR. Only one strain showed a slow reaction with the latex agglutination test. The three methods tested revealed 100% sensitivity and specificity.

Discussion

The present study aimed at evaluate the usefulness of the application of three rapid methods for the early detection of MRSA strains in the routine laboratory practice. All methods evaluated showed 100% sensitivity and specificity. It should however be noted that the latex agglutination test showed one slow reactive strain, that would have been missed if the initial manufacturer recommended cut-off value of three minutes had been applied; this cut-off value has been recently enlarged by the manufacturer to ten minutes.

The latex agglutination test is very simple and quick to perform taking four hours to complete. The Veloge...

References