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

Maria João Soares[a], Carlos Soares[a], Ana Constança Mendes[b], Maria Luís Guimarães[b], José Manuel Cabeda[b] y José Manuel Amorim[a]

[a]Microbiology Service. [b]Molecular Biology Unit. Hospital Geral de Santo António. Porto. Portugal.

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[1-3]. Given the clinical and epidemiological importance of MRSA isolates at the hospital setting, the rapid identification of MRSA is of paramount importance[4].

Methicillin resistance in *Staphylococcus aureus* is due to the hiperproduction of the low-affinity penicillin-binding protein PBP2a, coded by the *mecA* gene whose presence is not detected in methicillin sensitive *S. aureus* strains[5-8].

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[4,6,9,10].

The *femA* gene, highly conserved among Staphylococci, has been used for the identification of Staphylococci species[11,12].

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:

1. MRSA screen. A latex agglutination test using monoclonal anti-PBP2a.
2. BBL crystal MRSA ID System. This test uses an oxygen sensitive fluorofore, 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 hibridization 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 de 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 (Innogenetics, Japan), BBL Crystal MRSA ID (Becton Dickinson, USA) and Velogene Genomic ID Assay (Alexon-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

Correspondencia: Dra. M.J. Soares.
Microbiology Service. Hospital Geral de Santo António.
Largo da Escola Médica 4099. 0011 Porto. Portugal.
Correo electrónico: jcabeda@mail.telepac.pt

Manuscrito recibido el 25-3-2003; aceptado el 20-10-2003.

obtained after centrifugation (7,500 rpm; 10 min) was ressuspended in 300 µl Tris-EDTA buffer (Qbiogene, USA) and was lised with 10 µl of 5mg/ml lysostaphin (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* genes was performed as previously described[11] with a few modifications: 25 pmol of each primer M1 and M2 (*mecA* gene), 100 pmol of each primer F1 and F2 (*femA* gene), 2.5 mM $MgCl_2$, 2U DyNAzyme 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 686bp 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 aglutination 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 (15 minutes) to perform, being also the less expensive of the three methods assayed. BBL crystal MRSA ID, albeit easy to perform takes four hours to complete. The Velogene Genomic assay is the most expensive and difficult to perform providing results in approximately 90 minutes.

Though the good correlation observed between MRSA-Screen, BBL Crystal MRSA ID, Velogene Genomic ID Assay and the detection of *mecA* by PCR, the ease of use, turnaround time and price of MRSA-Screen were important for its selection as the routine method for MRSA screening.

References

1. Qadri SM, Yoshio Ueno, Imambaccus H, Almodovar E. Rapid detection of methicillin-resistant *Staphylococcus aureus* by Crystal MRSA ID System. J Clin Microbiol 1994;32:1830-2.
2. Voss A, Milatovich D, Wallrauch-Schwarz C, Rosdahl VT, Braveny I. Methicillin-Resistant *Staphylococcus aureus* in Europe. Eur J Clin Microbiol Infect Dis 1994;13:50-5.
3. Panlilio AL, Culver DH, Gaynes RP, Banerjee S, Henderson TS, Tolson JS, et al. Methicillin-Resistant *Staphylococcus aureus* in U.S. Hospitals, 1975-1991. Infect Control Hosp Epidemiol 1992;13:582-6.
4. Louie L, Matsumura SO, Choi E, Louie M, Simor AE. Evaluation of three rapid methods for detection of methicillin resistance in *Staphylococcus aureus*. J Clin Microbiol 2000;38:2170-3.
5. Willem B, Van Leeuwen, Van Pelt C, Luijendijk A, Verbrugh HA, Wil HF. Goessens. Rapid detection of methicillin resistance in *Staphylococcus aureus* isolates by the MRSA-Screen Latex Agglutination Test. J Clin Microbiol 1999;37:3029-30.
6. Cavassini M, Wenger A, Jaton K, Blanc DS, Bille J. Evaluation of MRSA-Screen, a simple anti PBP2a slide latex agglutination kit, for rapid detecction of methicillin resistance in *Staphylococcus aureus*. J Clin Micobiol 1999;37:1591-4.
7. Chambers HF. Methicilllin resistance in Staphylococci: molecular and biochemical basis and clinical implications. Clin Microbiol Rev 1997;10:781-91.
8. Felten A, Bernadette G, Lagrange PH, Isabelle C. Evaluation of three techniques for detection of low-level methicillin-resistant *Staphylococcus aureus* (MRSA): a disk diffusion method with cefoxitin and moxalactam, the Vitek 2 system, and the MRSA-Screen latex agglutination test. J Clin Microbiol 2002;40:2766-71.
9. Hussain Z, Luba S, Shaunalee G, Susan L, Viivi F, Robert L. Rapid detection of *mecA* positive and *mecA* negative coagulase-negative Staphylococci by an anti-penicillin binding protein 2a slide latex agglutination test. J Clin Microbiol 2000;38:2051-4.
10. Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S. Identification of methicillin-resistant strains of staphylococci by polimersae chain reaction. J Clin Microbiol 1991;29:2240-4.
11. Vannuffel P, Gigi J, Ezzedine H, Vandercam B, Delmee M, Wauters G, et al. Specific detection of methicillin-resistant *Staphylococcus* species by multiplex PCR. J Clin Microbiol 1995;33:2864-7.
12. Hamels S, Gala JL, Dufour S, Vannuffel P, Zammatteo N, Remacle J. Consensus PCR and Microarray for diagnosis of the genus *Staphylococcus*, species, and methicillin resistance. Biotecniques 2001;31:1364-72.