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ePoster Viewing

Molecular bacteriology

"Evaluation of Simplexa™MRSA Direct (Focus Diagnostics) on a large collection of *mecA*- and *mecC*-positive MRSA representing the major MRSA clones circulating throughout the world"

C. Dupieux[1,2], H. Meugnier[1,2], A. Garriga[1,2], J. Tasse[1,2], A. Tristan[1,2], M. Bes[1,2], F. Vandenesch[1,2], F. Laurent[1,2]

[1]*French National Reference Centre for Staphylococci - Hospices Civils de Lyon, Lyon, France*

[2]*International Centre for Infectiology Research - INSERM U1111, Lyon, France*

Introduction. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major pathogen responsible for nosocomial infections. Early screening for MRSA carriage of patients is pivotal to limit infections and transmission. Several PCR assays have been designed to ensure rapid and reliable detection of MRSA in nasal swabs. They classically target i) the junction between *orfX* and SCC*mec* cassette, ii) a gene specific to *S. aureus*, and iii) for some assays the *mecA* gene itself. Recently, identification of a variant of *mecA* gene, named *mecC* and presenting less than 70% DNA homology, has been reported and complexifies even more the MRSA detection due to misdiagnosis related to mismatch in primers targeting *mecA* gene. In this context, using a very diverse collection of MRSA clinical isolates, we evaluated a new commercial real-time PCR (Simplexa MRSA™ Direct) able to detect both *mecA* and *mecC* genes.

Materials and Methods. One hundred and forty-eight clinical MRSA and 4 MSSA (used as controls) were included. MRSA strains have been previously extensively characterized (*mecA*/*mecC* PCR, *agr* typing, DNA microarray (Alere/Clondiag)) and selected to be representative of the major worldwide-circulating MRSA clones: they covered 35 clonal complexes and more than 70 *spa*-types, and included 25 *mecC*-positive strains.

The Simplexa™ MRSA Direct assay is a real-time PCR that detects conserved regions of *S. aureus* genome (*spa* gene) and methicillin resistance genes (*mecA* and *mecC*). The software automatically compares the results (including Ct) of both targets to identify if MRSA or MSSA are present. The 1-hour assay was performed on a Direct Amplification Disc (8 tests) and the 3M™ Integrated Cycler with Integrated Cycler Studio Software version 5.0.

Results. All clinical isolates were accurately classified as methicillin susceptible (n=4) or methicillin resistant (n=148). All isolates were correctly identified as *S. aureus*, except one for which no *spa*-gene amplification was obtained. A 99.34 % agreement was so reported for the Simplexa MRSA™ Direct assay.

Conclusion. The use of the Simplexa™ MRSA Direct assay proved to be very simple and fast directly from isolated colonies. Using a large collection of strains representing the major MRSA clones circulating throughout the world, we obtained a 99.34% agreement with the reference methods; a single discrepancy has been observed with a MRSA isolate for which *spa* gene was not detected. Attempts of sequencing of the *spa* gene has shown that this strain likely belongs to the very rare strains of *S. aureus* lacking *spa* gene or partly deleted in *spa* gene.