

ABSTRACT

Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is responsible for localized or systemic infection in hospitalized patients. MRSA colonized children are at greater risk of developing "difficult to treat infections" especially because impaired or weakened immune system. Sampling collection devices and MRSA detection assays are both critical steps of the MRSA investigation procedures. Moreover despite the high incidence of MRSA there is no guideline to recommend microbiological workflow to detect MRSA carriage.

The objective of this study was to validate the New Copan ESwab collection devices (consisting of a tube with 1 ml of Amies medium and a flocked swab) **and the GenXpert MRSA/SA Nasal Assay (Cepheid)**, a multiplex real-time PCR assay that simultaneously detects *S. aureus*, *mecA* gene and staphylococcal cassette chromosome (SCCmec) in less than in one hour, to optimize MRSA screening "from collection to detection" in children admitted to the "Bambino Gesù" Children Hospital screened for *S. aureus* colonization

Methods: Nasal swabs were collected from 1040 paediatric patients at admission and discharge at the Medical Department and tested for both bacterial culture and molecular assay from the same specimen. One aliquot of each ESwab sample was cultured in Chapman and MRSA chromogenic agar. Another aliquot of ESwab was tested directly in the Xpert MRSA/SA Nasal Assay, to genotypically differentiate MSSA and MRSA strains. After 24h growth, *S. aureus* colonies were identified by using MALDI-TOF Mass Spectrometry. Confirmation of identification and oxacillin/cefoxitin testing was performed by the Vitek2 systems.

Results: In the 1634 nasal swab, *S. aureus* was found in 356 samples. The Vitek2 identified 21 MRSA and 335 MSSA. Instead the genXpert assay detected all 356 *S. aureus* positive of which 325 (91.29%) were MSSA, 13 MRSA (3.65%), 14 empty cassette (SCC positive and *mecA* negative) (3.37%) and 4 *S. aureus* SCC negative and *mecA* positive (1.12%).

Conclusion: Both cultures and molecular testing results were obtained from the same ESwab without any interference with the molecular assay. Availability of a "simple" sample collection swabs able to be used as well with phenotypical as molecular methods allows to optimize the MRSA detection strategy. This study demonstrated that the Copan ESwab sample collection is compatible with the Xpert MRSA/SA Nasal Assay and, allows MRSA screening "from collection to detection" from the same clinical sample collection.

REFERENCE

- Stefani S, Varaldo PE. "Epidemiology of methicillin-resistant staphylococci in Europe". *Clin Microbiol Infect.* 9(12):1179-86. 2003
- Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, Nouwen JL. "The role of nasal carriage in *Staphylococcus aureus* infections". *Lancet Infect Dis.*, 5(12):751-62. 2005
- Verhoeven P, Grattard F, Carricajo A, Pozzetto B, Berthelot P. "Better detection of *Staphylococcus aureus* nasal carriage by use of nylon flocked swabs". *J Clin Microbiol.* 48(11):4242-4. 2010
- Shurland S, Zhan M, Bradham DD, Roghmann MC. "Comparison of mortality risk associated with bacteremia due to methicillin-resistant and methicillin-susceptible *Staphylococcus aureus*". *Infect Control Hosp Epidemiol.* 28(3):273-9. 2007
- Wolk DM, Picton E, Johnson D, Davis T, Pancholi P, Ginocchio CC, Finegold S, Welch DF, de Boer M, Fuller D, Solomon MC, Rogers B, Mehta MS and Peterson LR. "Multicenter Evaluation of the Cepheid Xpert Methicillin-Resistant *Staphylococcus aureus* (MRSA) Test as a Rapid Screening Method for Detection of MRSA in Nares". *J Clin Microbiol.* 47(3): 758-764. 2009

OBJECTIVES

Methicillin-resistant *Staphylococcus aureus* (MRSA) have emerged as major nosocomial pathogens during the past two decades and it is resistant not only to all b-lactam but also to a wide range of other antibiotics (Stefani et al, 2003). About 20% of individuals are persistent *S. aureus* nasal carrier, and anterior nares of the nose is the main ecological niche where the bacteria resides (Wertheim et al., 2005). Despite a great number of informations about epidemiology of *S. aureus* and his mechanism of resistance we still debate the best microbiological procedure for a rapid and accurate identification of MRSA strains.

The objectives of this study were:

- To validate the New Copan ESwab collection devices (consisting of a tube with 1.0 ml of Amies medium and a flocked swab) and the GenXpert MRSA/SA Nasal Assay (Cepheid), a multiplex real-time PCR assay that simultaneously detects *S. aureus*, *mecA* gene and staphylococcal cassette chromosome (SCCmec) in less than in one hour
- To optimize MRSA screening "from collection to detection" in children admitted to the "Bambino Gesù" Children Hospital screened for *S. aureus* colonization

METHODS

In this study, 1040 nasal swabs were collected from paediatric patients, admitted at the Medical Department of "Bambino Gesù" Children Hospital and screened for MRSA detection from January to June 2010. Patients hospitalized for invasive surgical procedure or admitted at Intensive Care Unit (ICU) were excluded from this study.

•Sample collection was performed with the new collection and transport device ESwab (Copan Italia SpA, Brescia-Italy), consisting of a flocked swab and a tube with 1.0 ml of Amies liquid transport medium, reported to have a high efficiency for recovering bacteria (Verhoeven et al., 2010).

•All specimens were tested simultaneously by traditional cultures and by the molecular Xpert MRSA assay (Fig.1).

The primers and probes of the new Xpert MRSA/SA Nasal assay detect proprietary sequences for the staphylococcal protein A (*spa*), the gene for methicillin/oxacillin resistance (*mecA*), and the staphylococcal cassette chromosome (SCCmec). The sample processing control (SPC) verifies the adequate processing of the target bacteria and to monitor the presence of inhibitors in the PCR reaction.

For each nasal sample:

- One aliquot (10 µl) of ESwab liquid medium was streaked onto Chapman (bioMérieux, France) and MRSA chromogenic agar (Bio-Rad, USA), and incubated at 37 C for 18-24h. Colonies suggestive of *S. aureus* were subcultured onto 5% sheep blood agar and identified by using MALDI-TOF Mass Spectrometry. Confirmation of identification and oxacillin/cefoxitin resistance was tested by the Vitek2 systems (bioMérieux).
- One aliquot (200 µl) ESwab liquid medium was tested using the Xpert MRSA/SA Nasal according to the manufacturer's instructions.

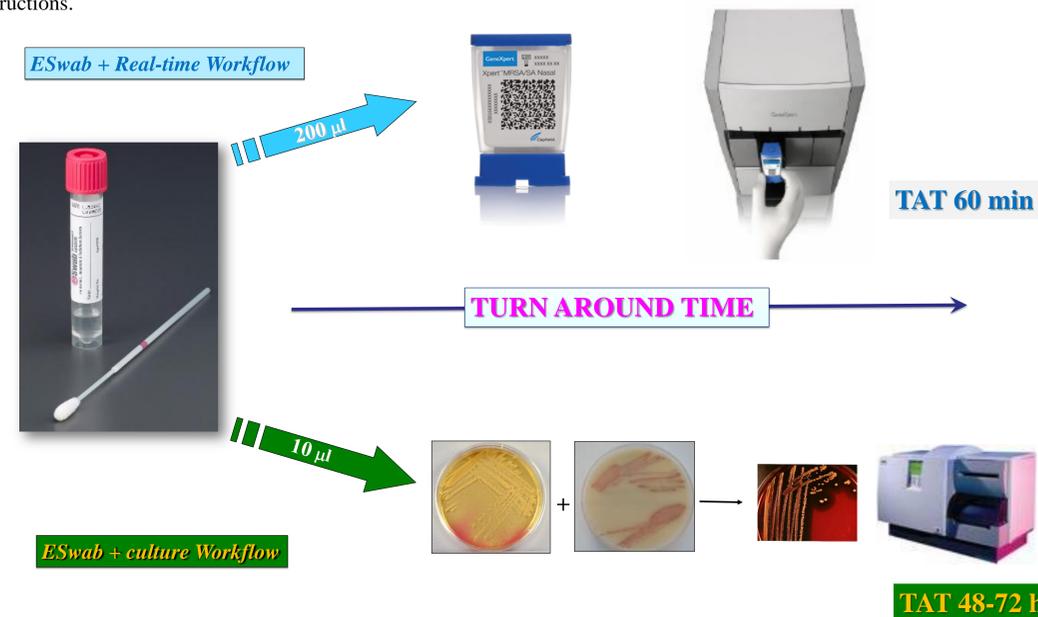


Fig.1 Study workflow

RESULTS

We investigated 1634 nasal swabs collected from 1040 paediatric patients

Xpert MRSA/SA Nasal assay allowed to detect 356 *S. aureus*

- ✓ 343 SA positive/MRSA negative:
 - 325 MSSA
 - 14 SCC positive and *mecA* negative (*Empty cassette variant*)
 - 4 SA positive/SCC negative and *mecA* positive (*SCC modified variant*)
- ✓ 13 SA positive/MRSA positive = MRSA

The Vitek2 system identified 356 *S. aureus*

- 335 cefoxitin negative = MSSA
- 21 cefoxitin positive = MRSA

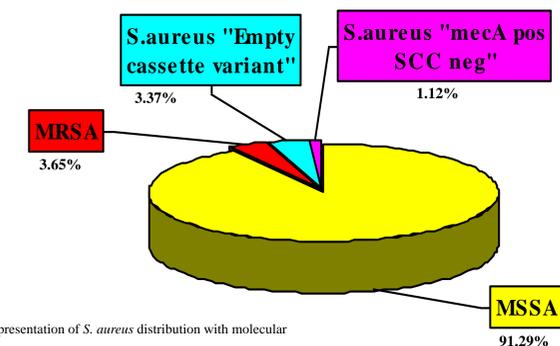


Fig.2. Graphic representation of *S. aureus* distribution with molecular

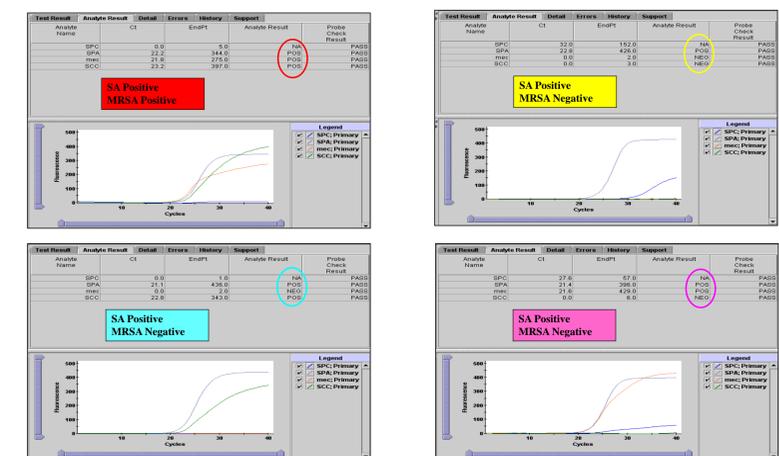


Fig.3. Xpert MRSA/SA Nasal results presentation

CONCLUSION

Rapid and accurate detection of MRSA carriers is very important because hospital-acquired infections, often due to antibiotic-resistant strains, have been associated with increased morbidity and mortality (Shurland et al., 2007).

We conducted a study to assess the clinical performance of the new Copan ESwab device for screening MRSA detection by using simultaneously both methods and we observed:

- 1) the total agreement between cultures and molecular assay for recovering SA;
- 2) the absence of interference with Real-time PCR using the liquid transport medium of Copan ESwab;
- 3) the ability of the XperMRSA/SA Nasal Assay to provide additional informations about SA variants not detectable by phenotypical methods.

This study demonstrate that the Copan ESwab collection and transport device is compatible with the new XperMRSA/SA Nasal Assay.

The combined use of Copan ESwab and XperMRSA/SA Nasal Assay allows MRSA screening "from collection to detection" can be use as a replacement for phenotypical method specially in case if a reduced TAT is mandatory.