Detection of Methicillin-Resistant Staphylococcus aureus using Magnetic Immunocapture Combined with Real-Time Multiplex qPCR Analysis

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Abstract (revised)

Rapid and efficient identification of methicillin-resistant Staphylococcus aureus (MRSA) carriage can definitively help to improve infection control strategies such as isolation of patients and decontamination procedures. Molecular diagnostics can be completed in few hours in contrast with conventional culture techniques which usually take 48 to 72h. This study aims at evaluating the performance of magnetic immunocapture combined with real-time qPCR on a collection of healthcare- and community-associated isolates, and on clinical samples (swabs). Immunocapture of S. aureus was performed using MRSAdembeads (Ademtech). Immunocaptured bacteria were used with an enzymatic cocktail and bacterial qPCR was performed using Smart-Adembeads (Ademtech). Real-time multiplex qPCR analyses were run on a 7500 Fast System SDS apparatus (Applied Biosystems) in the presence of Smart-Adembeads with specific primers and TaqMan probes targeting femA gene from S. aureus and S. epidermidis, and mecA gene. Detection of MRSA was investigated from model mixtures of bacteria and from clinical samples in 200µL PBS-BSA 1%. Patient小心临床试样的选择是提高敏感性的关键。

Model Study. Initial suspensions were prepared in NaC 0.9% from isolated colonies of bacteria selected from 24h agar plates (10^6 CFU/mL). Diluted suspensions were then prepared in 200µL PBS-BSA 1%. Immunocapture composition, recovery and non-specific capture were evaluated from agar plates counts after incubation at 37°C for 24h. Clinical Study. Bacteria were collected from Amies-Agar Transport Swabs (Copan) and suspended in 200µL PBS-BSA 1%. Diluted suspensions were then prepared in 200µL PBS-BSA 1%. Inoculum composition, recovery and non-specific capture were evaluated from agar plates counts after incubation at 37°C for 24h. Immunocapture

Immunoassay

MRSAdembeads (Ademtech) were synthesized by immobilizing anti- S. aureus antibodies covalently linked to magnetic particles. The capture efficiency was evaluated on a wide variety of S. aureus strains from S. aureus/ S. epidermidis mixtures. The presence of other common-encounter bacteria (Staphyloccoccus haemolyticus, Pseudomonas aeruginosa, Proteus vulgaris and Enterococcus faecalis) in nasal swab was investigated and did not affect the capture efficiency of S. aureus.

Molecular detection of MRSA from spiked samples

Clinical swabs were spiked with N315 isolates and processed following the magnetic immunocapture-coupled PCR procedure. Examples of qPCR analyses

Clinical Study : Detection of MRSA from clinical samples

239 nasal and oropharyngeal swabs collected from Amies-Agar Transport Swabs were screened for MRSA with the reference culture method and with qMRSA-Assay. Culture method. Swabs were used to inoculate the selective agar MRSA-ID and then suspended in 2mL CS-broth (brain-heart infusion with 10% calf serum) and PBS-BSA 1% as a backup media. Plates were incubated at 37°C and read after 24h.

Conclusions

Infections due to MRSA are frequent and represent an economical burden, requiring utilisation of barrier drugs. Thus, rapid detection and identification of MRSA is an absolute prerequisite to adopt prompt isolation measures. Until recently, molecular assays dedicated to MRSA identification were based on the utilisation of selective growth media, which are time-consuming and preclude same-day diagnosis. However, molecular assays based on targeted nucleic acid amplification have proven rapid, affordable and successful in terms of sensitivity and specificity. We have developed a novel molecular assay using magnetic particles allowing detection of MRSA from mixed flora samples. Compared to other commercial molecular assays, qMRSA assay can efficiently lower the number of false-positive results and is not sensitive to epidemiological variability by directly detecting the mecA gene and its target by using a S. aureus-specific femA gene.

References


Data

<table>
<thead>
<tr>
<th>MRSA</th>
<th>HA-MRSA</th>
<th>CA-MRSA</th>
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</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>98%</td>
<td>97%</td>
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S. aureus was used as a control strain.

Efficient recovery of S. aureus while keeping non-specific capture low (%)

This novel assay involving magnetic immunocapture, enzymatic lysis and DNA purification enhances the recovery of DNA available for real-time multiplex qPCR and therefore improves the limit of sensitivity: 10 CFU/plate were thus successfully detected.

Besides, by using magnetic particles, the procedure can be easily automated allowing high-throughput analyses for on-admission screening.

Introduction

Staphylococcus aureus is a major pathogen responsible for both healthcare- and community-associated infections. The rapid detection of inpatients carrying methicillin-resistant S. aureus (MRSA) could contribute to minimise MRSA transmission and may even be cost-beneficial (1, 2). Recently, our group showed that the "same-day diagnosis" of MRSA contributed to the reduction of nosocomial MRSA infections in a medical intensive care unit when linked with appropriate isolation measures (3). To date, the "gold standard" method for MRSA identification relies on culture (+) and provides results in approximately 48-72h whereas molecular methods cupboard complete detection strategies by providing rapid and sensitive detection. The mecA gene, originating from a mobile genetic element (named SCCmec) inevitably inserted in the oriV gene of methicillin-resistant Staphylococcus aureus is the genetic basis of methicillin-resistance. Additionally, the high similarity between mecA sequences in the different staphylococcal species, precludes identification of MRSA using mecA as a single identification target (4). This study reports the use of a novel immuno-qPCR procedure allowing rapid detection of MRSA from mixed flora samples. The procedure consists in a direct one-step enrichment of S. aureus present in either nasal or oral swabs, followed by DNA purification and immunocapture and their identification by a triplex qPCR. The specificity of MRSA identification is based on the quantitative correlation of the mecA gene and that of the S. aureus-specific femA signal, a probe that does not cross-react with other bacterial species, including S. epidermidis.

Molecular detection of MRSA from spiked samples

Suspended of S. aureus/ S. epidermidis mixtures were processed following the magnetic immunocapture-coupled PCR procedure.

Examples of qPCR analyses

Clinical swabs were spiked with N315 isolates and processed following the magnetic immunocapture-coupled PCR procedure. Examples of qPCR analyses

Method

- qMRSA
- Positive
- Negative
- Total
- Culture
- MRSA prevalence =10% (from the reference culture method)
- From the 14 culture-negative but qMRSA-positive specimens, 7 specimens were found to be culture-positive upon further investigations. 3 specimens originated from heavily contaminated patients, resulting in a total of 59 culture and qMRSA-positive specimens out of a total 60 culture-positive specimens.
- The false-negative specimen was found to be highly contaminated with MSSA and contained two MRSE. MRSA detection clearly failed by qPCR. MRSA was only detected after CS-broth enrichment.
- No PCR inhibition was noted for all the specimens.

Conclusion

This study reports the use of a novel immuno-qPCR procedure allowing rapid detection of MRSA from mixed flora samples. The procedure consists in a direct one-step enrichment of S. aureus present in either nasal or oral swabs, followed by DNA purification and immunocapture and their identification by a triplex qPCR. The specificity of MRSA identification is based on the quantitative correlation of the mecA gene and that of the S. aureus-specific femA signal, a probe that does not cross-react with other bacterial species, including S. epidermidis.