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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 definitely 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 lysed with an enzymatic cocktail and bacterial gDNA was purified 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* genes 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 clinical specimens were screened for MRSA following the immunocapture-coupled PCR procedure (qMRSA) and compared to a reference method using standard culture and enrichment broth (MRSA ID (bioMérieux) and CS-broth). All processed samples were nasal or nasal-inguinal swabs. Sensitivity of the procedure allows identification of all culture-positive specimens. The limit of sensitivity was 10 CFU/swab. About 5% of culture-negative specimens were found to be qMRSA-positive. Most of discrepant results corresponds to freshly-decontaminated previously-known MRSA carriers or were found to be culture-positive upon further investigation.

A novel rapid and simple procedure using magnetic particles was developed for identification of MRSA carriage from clinical swabs. The efficiency of the magnetic immunocapture combined with real-time qPCR analysis was validated on healthcare- and community-associated MRSA strains.

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 minimize MRSA transmission and may even be cost-beneficial (1, 2). Recently, our group showed that the "same-day detection" of MRSA contributed to the reduction of nosocomial MRSA infections in a medical intensive care unit when linked with appropriate isolation measures (3).

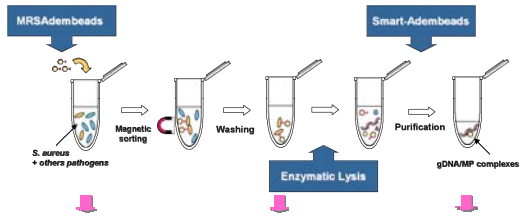
To date, the "gold standard" method for MRSA identification relies on cultures (4) and provides results in approximately 48-72h whereas molecular methods outperform conventional detection strategies by providing rapid and sensitive detection. The *mecA* gene, originating from a mobile genetic element (named SCC*mec*) invariably inserted in the *orfX* gene of methicillin-resistant staphylococci 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 (5).

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 inguinal swabs, followed by DNA purification of immunocaptured bacteria 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*.

Principle of qMRSA Assay and Methodology

Model Study. Initial suspensions were prepared in NaCl 0.9% from isolated colonies of bacteria selected from 24h agar plates (10^8 CFU/mL). 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.

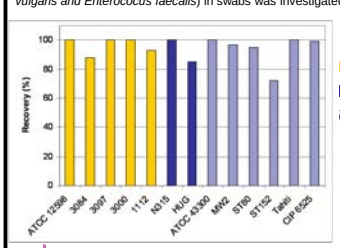
Clinical Study. Bacteria were collected from Amies-Agar Transport Swabs (Copan) and suspended in 200µL PBS-BSA 1%.



Immunocapture

MRSAdemBeads (Ademtech) were synthesized by immobilizing anti-*S. aureus* mAb on carboxylic 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 commonly-encountered bacteria (*Staphylococcus haemolyticus*, *Pseudomonas aeruginosa*, *Proteus vulgaris and Enterococus faecalis*) in swabs was investigated and did not affect the immunocapture efficiency.



Efficient recovery of *S. aureus* while keeping non-specific capture low (< 1%)

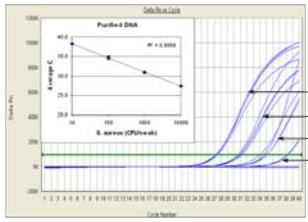
Reference	Type	Origin
ATCC12598	MSSA	US
30.84	MSSA	FR
30.97	MSSA	FR
30.00	MSSA	FR
11.12	MSSA	FR
N315	HA-MRSA	US
HUG	HA-MRSA	CH
ATCC43300	CA-MRSA	US
MW2	CA-MRSA	US
ST80	CA-MRSA	Europe
ST152	CA-MRSA	Kosovo
Tahiti	CA-MRSA	Polynesia
CIP 6525	CA-MRSA	FR

Molecular detection of MRSA from spiked samples

gDNA was extracted and purified from immunocaptured bacteria using an enzymatic cocktail and Smart-Adembeads (Ademtech). 3µL of 50µL purified DNA were then analyzed by real-time triplex qPCR targeting *mecA*, *S. aureus*- and *S. epidermidis*-specific *femA* genes and using specific TaqMan probes.

❑ **Detection of MSSA from model inoculums**

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

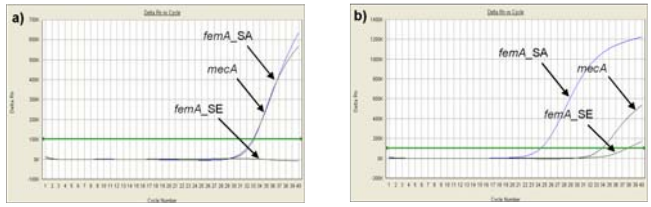


- Enzymatic lysis combined with DNA purification enhances DNA recovery
- Good reproducibility
- Excellent linearity
- Limit of sensitivity close to 10 CFU/swab
- Smart-Adembeads fully compatible with real-time qPCR analyses

❑ **Detection of MRSA from spiked swabs**

Clinical swabs were spiked with N315 isolate and processed following the magnetic immunocapture-coupled PCR procedure.

Examples of qPCR analyses



Ct (*femA*_SA) = Ct (*mecA*)
*femA*_SE was not detected → **MRSA**

Ct (*femA*_SA) - Ct (*mecA*) < 0
Ct (*mecA*) – Ct (*femA*_SE) < 0 → **MRSA + MSSA**

Clinical Study : Detection of MRSA from clinical samples

338 nasal and nasal/inguinal swab samples collected with Amies-Agar Transport Swab 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 10µg colistin/mL and 2.5% NaCl) as a backup media. Plates were incubated at 35°C and read after 24h.

Method		Culture		
		Positive	Negative	Total
qMRSA Assay	Positive	52	14	66
	Negative	1	271	273
	Total	53	285	338

MRSA prevalence =16%
(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 freshly decontaminated 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 few MRSE, MRSA detection clearly failed by qPCR. MRSA was only detected after CS-broth enrichment.

✓ **No PCR inhibition was noted for all the specimens**.

- Sensitivity = 98.1%
- Specificity = 97.5%
- PPV = 89.4%
- NPV = 99.6%

Conclusions

Infections due to MRSA are frequent and represent an economical burden, requiring utilisation of last barrier drugs. Thus, rapid detection and identification of MRSA is an absolute prerequisite to adopt prompt isolation measures. Until recently, microbiological methods 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 origin by using a *S. aureus*-specific *femA* gene.

The overall procedure leads to successful identification of MRSA-positive specimens in 2-3h with sensitivity and specificity of 98% and 97.5%, respectively. In addition, the determined NPV value appears especially interesting for the use of the molecular assay in high-prevalence area.

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

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

References

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