# Evaluation of 2 real-time PCR assays for the investigation of mecA gene in clinical isolates of MRSA in Western Saudi Arabia

***Baraa T. Al-Haj-Hussein,*** *MSc, FIBMS,* ***Mohammed A. Al-Shehri,*** *BSc,* ***Esam A. Azhar,*** *MSc, PhD,* ***Ibraheem M. Ashankyty,*** *MSEd, PhD,* ***Abimbola O. Osoba,*** *MD (Hons), FRC (Path).*

## ABSTRACT

**Objective:** In diagnostic microbiology laboratories, Methicillin resistant *Staphylococcus aureus* (MRSA) is identified by positive coagulase test and positive deoxyribonuclease (DNase) activity followed by demonstration of oxacillin resistance on susceptibility testing on agar plate. This usually takes an approximately 48-72 hours. The purpose of this study is to evaluate 2 real-time polymerase chain reaction (PCR) assays for the presence of mecA gene in a population of MRSA strains circulating in Jeddah, Western Saudi Arabia, in order to determine their usefulness in the speedy diagnosis of MRSA in our clinical setting and their contribution to optimal patient management.

**Methods:** Ninety MRSA isolates obtained from clinical samples were identified by using conventional methods. They were collected between February 2004 and August 2004, from 2 major hospitals in Jeddah; King Abdul-Aziz University Hospital, Jeddah (50 strains) and King Khalid National Guard Hospital, Jeddah (40 strains). All isolates were confirmed as MRSA using Gram stain, catalase and coagulase activity, confirmatory DNAse activity and Kirby Bauer disc diffusion method with resistance to oxacillin by the agar disc method. The DNA extract was tested by 2 assays. The first was the commercial IVD Roche kit, which detects the mecA gene using the Light Cycler system. The other method employs multiplex PCR which detects As442 fragment and mecA optimized for the Smart Cycler system (Cephied). The length of time taken to perform the assays was recorded.

**Results:** All isolates were positive for Sa442 fragment and the coa gene specific for *Staphylococcus aureus (S. aureus)*. However, 88/90 isolates (97.7%) tested were positive for mecA gene with both systems. The amplification, detection and melting curve analysis took 59.2 minutes for 32 samples on the Light Cycler and 46.7 minutes for 16 samples on the Smart Cycler.

**Conclusion:** The 2 methods studied were equally specific and sensitive for the detection of mecA gene in confirmed *S. aureus* isolates and capable of identifying MRSA much earlier than conventional methods. The detection of 2 targets in the multiplex PCR assay reduces the 2-hour time required for DNase testing and may be used as a primary screening test for the detection of MRSA in clinical samples, such as blood cultures and sterile body fluids.

**Saudi Med J 2005; Vol. 26 (5): 759-762**

Methicillin-resistant *Staphylococcus aureus* (MRSA) is now recognized as a serious and common cause of nosocomial and community acquired infections world wide.[1-4] Infected persons may be simply colonized or develop infections ranging from wound infections to severe infections such as bacteremia and septicemia. The epidemiology of MRSA in Saudi Arabia has varied from a very low to high rates in different areas of the Kingdom. Madani et al[5] in 1998 reported that

From the Molecular Biology Unit, Division of Microbiology (Al-Haj-Hussein, Al-Shehri, Osoba), King Khalid National Guard Hospital and the Faculty of Applied Medical Sciences (Azhar, Ashankyty), King Abdul-Aziz University, Jeddah, *Kingdom of Saudi Arabia.*

Received 13th December 2004. Accepted for publication in final form 27th February 2005.

Address correspondence and reprint request to: Prof. Abimbola O. Osoba, Division of Microbiology, King Khalid National Guard Hospital, PO Box 9515, Jeddah. 21423, *Kingdom of Saudi Arabia*. Tel. +966 (2) 6240000. Fax. +966 (2) 6247444. E-mail: osobaao@ngha.med.sa

222 (33%) of 673 *Staphylococcus aureus (S. aureus*) isolates collected from 2 tertiary health care centers in Jeddah were MRSA.[5] In Abha (Southern part of Saudi Arabia), it was reported that MRSA was isolated from 5.1% and 18.3% of non-hospital and hospital patients, while 61% of *S. aureus* isolates from infected patients were MRSA.[6] However in the Eastern province, it was reported that of 1,096 *S. aureus* identified, 92 (8.4%) were MRSA.[4] In our laboratory in 2003, out of 1420 strains of *S. aureus* isolated, 440 (31%) were MRSA, which indicates that MRSA were highly prevalent in Jeddah area, and there is evidence that MRSA strains were also circulating in the community.[7]

In clinical laboratories, MRSA is identified by a positive coagulase test, catalase test and deoxyribonuclease (DNase) activity followed by oxacillin resistance on disc susceptibility testing. The turn around time for such testing is 48-72 hours. If the patient is suspected to be colonized or infected, the patient will be isolated in a single room. If however the patient is not suspected to be colonized by MRSA, the patient will continue to spread the organism to the contacts and may be the source of an outbreak, which may be costly to the institution. Consequently, in our hospital as well as in some hospitals, new admissions to the wards and particularly in the intensive care units are screened routinely for colonization for MRSA at some cost to the institution. As a result of the importance of early detection of MRSA in colonized or infected patients, rapid molecular methods for MRSA have been developed, with the hope of early detection to reduce transmission and forestall an outbreak. These methods detect coa, the gene pathognomonic for *S. aureus* and mecA gene, which encodes for methicillin resistance in *Staphylococci*, as well as Sa442 and femB genes, which are also *S.aureus* specific.[1-3,8] Several studies have confirmed the high specificity and sensitivity of the Light Cycler PCR for the detection of mecA and Sa442 genes in *S.aureus*.[9,10] Several commercial PCR–based assay kits are now available that rapidly detect simultaneously mecA gene and femB genes in *S. aureus* in <2 hours.

The purpose of our study is to evaluate the 2 real time PCR assays for the presence of mecA gene in a population of MRSA strains circulating in Jeddah city and their usefulness in the speedy diagnosis of MRSA as well as their clinical impact in the control of MRSA colonization/infection.

Methods. Ninety MRSA isolates were collected from a variety of clinical samples obtained between February 2004 and August 2004, from 2 major hospitals in Jeddah, Western region of Saudi Arabia. Fifty of the strains were collected at the King Abdul-Aziz University Hospital (KAUH), Jeddah, while 40 were isolates from King Khalid National Guard Hospital (KKNGH), Jeddah. In both hospitals, all isolates were confirmed as *S. aureus* using the Gram stain, positive catalase and coagulase tests and specific surface characteristics.[10-12] Confirmation of an *S. aureus* as MRSA was carried out using DNase activity and Kirby-Bauer disc diffusion method with the demonstration of oxacillin resistance (**Figure 1**).[10-12]

After boiling the *S. aureus* strains in PBS for 20 minutes, bacterial DNA was obtained from the supernatant after centrifugation. All the 90 DNA extracts were tested by the two molecular biological assays. The first system used commercial IVD kit (Roche Diagnostics, GmbH, Roche Applied Science, Sandhofer Strasse 110, D-68305 Mannheim, Germany). This kit detects the mecA gene using the Light Cycler system in strains confirmed as *S. aureus* isolates. The other assay employs multiplex PCR which detects Sa442 fragment specific for *S. aureus* and mecA gene.[10] The primers and probes were made by TIB Molbiol, Berlin, Germany and optimized locally for the Smart Cycler system (Cephied, Sunnyvale, CA, USA). All assays were performed according to manufacturer's instructions. The details of the primers used are shown in **Table 1**. The Roche kit contains positive and negative internal controls for reliable interpretation of the results. Known methicillin sensitive *S. aureus* (MSSA) isolates and mecA positive MRSA isolates were used as negative and positive controls in the Cephied assay (TIB Molbiol) primer set. The length of time taken to perform the assays was recorded (**Table 2**).

Results. All isolates were positive for Sa442 fragment specific for *S. aureus* (**Figure 2**). While all KAUH isolates were positive for MecA gene (50/50), 38/40 isolates of KKNGH (95%) were positive for mecA gene. Overall, 88 (97.4%) of the 90 isolates tested positive for mecA gene with both systems. The amplification, detection and melting curve analysis with both systems took 59.2 minutes for a batch of 32 samples (1.85 min/sample) on the Light Cycler and 46.7 minutes for a batch of 16 samples (2.9 minutes/sample) on the Smart Cycler system (**Table 2**).

Discussion. Screening of high-risk patients, patient's contacts and hospital personnel for MRSA has been the major measure used for the prevention and control of MRSA outbreak. The control strategy of identification, isolation of patients, contact precautions, hand hygiene and treatment has been successfully used in many hospitals and in our hospital, to reduce the nosocomial colonization/infection due to MRSA.[13] There are several recommended methods for minimal

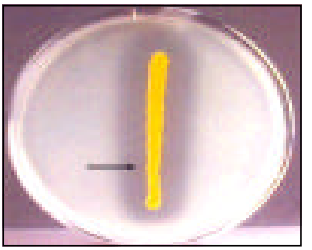

Figure 1 - A *Staphylococcus aureus* on a deoxyribonuclease (DNase) plate. The halo formed after adding HCl indicates positive DNAse activity.

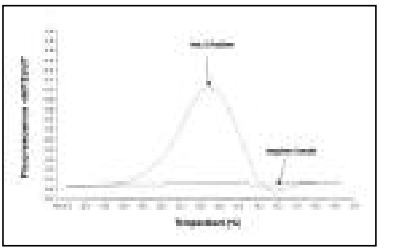

Figure 2 - The melting curve analysis produced by the light cycler. The positive peak refers to mecA specific temperature at $65^{0}$C.

Table 1 - Details of the primers and probers used for the assays.

| Primers and probes | | Length | GC % |
|---|---|---|---|
| ***Staphylococcus aureus mecA gene for penicillin-binding protein 2a*** | | | |
| *Primers* | | | |
| Mec-F | GGTGAAGATATACCAAGTGATTA | 23 | 34.7 |
| Mec-R | GTGAGGTGCGTTAATATTGC | 20 | 45.0 |
| *Probes* | | | |
| Mec-FL | CAGGTTACGGACAAGGTGAAATACTGATT | 29 | 41.4 |
| Mec-LC | ACCCAGTACAGATCCTTTCAATCTATAGCG | 30 | 43.3 |
| ***Staphylococcus aureus strain ATCC25923 clone pSa-442 Sau3AI fragment*** | | | |
| *Primers* | | | |
| Sa442-F | GTCGGTACACGATATTCTTCACG | 23 | 47.8 |
| Sa442-R | CTCTCGTATGACCAGCTTCGGTAC | 24 | 54.1 |
| *Probes* | | | |
| Sa442-FL | TACTGAAATCTCATTACGTTGCATCGGAA | 29 | 37.9 |
| Sa442-705 | ATTGTGTTCTGTATGTAAAAGCCGTCTTG | 29 | 37.9 |

Table 2 - Number of mecA positive and negative Methicillin resistant *Staphylococcus aureus* (MRSA) isolated from the 2 hospitals.

| MRSA isolates | King Abdul-Aziz University Hospital, Jeddah N=50 n (%) | King Khalid National Guard Hospital, Jeddah N=40 n (%) |
|---|---|---|
| MecA+ | 50 (100) | 38 (95) |
| MecA | 0 - | 2 (5) |

inhibitory concentration and disc diffusion testing of MRSA, due to the variable expression of resistance by different strains of MRSA. Some rapid or automated methods are available including latex agglutination techniques for the detection of PBP2a, which is responsible for the resistance to all ß-lactam antibiotics, encoded by mecA gene.[14] The "gold standard" method for the detection of resistance mediated by mecA gene now is the PCR, which is most commonly used in reference laboratories.[2,10,14] In the present study we have evaluated 2 real-time, rapid and sensitive PCR based assays, which will detect mecA gene from colonies of *S. aureus* isolated on culture, in less than one hour. The results are available at least 24 hours earlier than conventional cultures. The substantial time saved in the laboratory diagnosis of MRSA, is of great advantage in the early implementation of aggressive infection control measures and cost-effective in terms of hospitalization cost and isolation rooms. Although

this study was carried out on strains isolated on culture, there are now molecular assays for rapid detection of MRSA from patient screening swabs after the overnight incubation in broth. The specificity and sensitivity have been reported to be 100%, with a turnaround time of 3.5 hours.[2] Such systems would considerably reduce the time of diagnosis of MRSA to <4 hours from the time of collection of the swabs and thus allowing ample time for taking appropriate measures.

Methicillin-resistant *Staphylococcus aureus* septicemia is well known to cause severe morbidity and mortality if not treated early with intravenous glycopeptides, which are nephrotoxic and ototoxic.[15] Nowadays, several PCR based assays have been developed which detect MRSA directly in blood cultures with Gram-positive cocci in less than 2 hours, thus allowing prompt treatment with the appropriate antibiotics, as opposed to conventional culture methods which take 48 hours or more.[15] The molecular biological assays can be extended to urine, cerebrospinal fluid and synovial fluids in the future,[16] thereby improving patient management of MRSA infections in these locations.

In view of the high prevalence of MRSA in our locality, the introduction of screening of suspected or high risk patients with real-time PCR assays will not only allow earlier diagnosis but also limit the epidemiological damage of a colonized/infected patient. However, the initial cost of equipment and reagents is high and only clinical diagnostic laboratories, with established molecular biology laboratories or tertiary medical institutions can undertake such a venture. The complete protocol by these 2 methods can be completed in 2 hours, especially when testing direct samples, while culture based methods take 2-3 days, thus allowing for prompt and cost effective control measures, decolonization and treatment. They are highly sensitive, specific and have the potential of direct rapid diagnosis of MRSA in clinical specimens, plate cultures and even in patients undergoing anti-staphylococci therapy, in view of their low detection limit. They can be readily incorporated into the workflow of a clinical diagnostic laboratory along with the use of automated extraction systems. Recently, a new kit commercially known as IDI-MRSATM has been approved by the US Food and Drugs Administration and the Health Canada for use directly on clinical samples for the rapid detection of MRSA in a nasal swab specimen, with results available in about one hour.

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