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Rapid Identification of Methicillin Resistant *Staphylococcus aureus* Using Real Time PCR*

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ABSTRACT

Screening for colonization with methicillin resistant *Staphylococcus aureas* (MRSA) is a key aspect of infection control to limit the nosocomial spread of this organism. Current methods for the detection of MRSA in clinical microbiology laboratories using conventional methods is time consuming. In this research we are trying to evaluate the use of real time PCR for the detection of MRSA. The PCR assay was evaluated in clinical isolates of MRSA (n = 45) and methicillin susceptible *Staphylococcus aureas* MSSA (n = 10). The diagnostic values of the assay showed high sensitivity and specificity. This real-time PCR assay proved to be a fast, sensitive and specific tool for MRSA detection in a routine microbiological laboratory. Real-time PCR now is available in all laboratories so its use in identification of MRSA will help in shortening the period for MRSA identification and will help in the success of infection control programs in hospitals.

Keywords: Diagnosis; MRSA; Rapid Detection; Real-Time PCR; *Staphylococcus aureus*

1. Introduction

Staphylococci are ubiquitous colonizers of human or animal skin and mucous membranes causing a variety of syndromes. *Staphylococcus aureus* can cause nosocomial and community-acquired infections ranging from mild conditions, such as skin and soft tissue infections, to severe, life-threatening sepsis [1]. Strains of methicillin-resistant *S. aureus* (MRSA) were first detected in the early 1960s, shortly after methicillin came into clinical use [2]. During the past decade, an increase in the number of Methicillin resistant *Staphylococcus aureus* (MRSA) cases has been widespread globally among healthy community [3]. The mechanisms responsible for this resistance are diverse, and hundreds of resistance genes have been characterized in both Gram-negative and Gram-positive species. A core function of all bacteriology laboratories is to determine the antibiotic susceptibilities of bacterial isolates and to detect the resistance phenotypes conferred by these resistant genes. If a panel of agents in a particular antibiotic class is tested, the interpretative reading of the resulting patterns of resistance or susceptibility often suggests the underlying resistance mechanisms, predicts second-line drugs to be tested and provides extended knowledge for decisions in antimicrobial therapy as well as infection control. Methicillin resistance in Staphylococcus is mainly mediated by the overproduction of PBP2a, an additional modified penicillin binding protein (PBP) with low affinity for β-lactam antibiotics. The *mecA* gene, the structural determinant that encodes PBP2a, is therefore considered as a useful molecular marker of putative methicillin resistance in *S. aureus* [4]. The *mecA* gene can be detected by PCR, which is considered the gold-standard test to identify methicillin resistance [5]. The results of the conventional culture-based screening methods with cultivation, identification and antimicrobial susceptibility testing take 48 to 72 hr. Rapid detection of a specific resistance mechanism in a molecular test allows clinicians avoiding potentially inappropriate treatment options. To rapidly detect methicillin-resistant *Staphylococcus aureus* (MRSA) nasal colonization in patients, clinical microbiology laboratories should select either PCR methods or selective agar-based methods. Several chromogenic and differenttial MRSA selective agars have been shown to yield results within 18 to 24 hr [6]. In contrast, PCR methods can yield results in 2 to 3 hr. Antibiotic resistance genes provide important targets for molecular detection techniques [7]. Although molecular assays provide a quick identification of MRSA colonization as the results of the test can

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be available within a few hours, these methods are expensive. However, quick MRSA screening tests prevent unnecessary procedures as well as the spread of MRSA in case of colonized patients and thus reducing the overall hospital costs. It should be noticed that molecular biological techniques, such as DNA sequencing, or the use of DNA microarray technology [3], could provide more accurate identification and classification tool, but such techniques are difficult to apply in a routine clinical laboratory. A rapid, conventional and automated identification method based on phenotypic characters is a more practical approach for the daily clinical laboratory procedures.

The current study aimed to evaluate the use of real time PCR in detection of methicillin resistant *Staphylococcus aureus* (MRSA) and to trace the dynamics of genotypic changes and the shifts in the levels of susceptibility to antibiotics of MRSA isolated from patients in King Abdel Aziz Hospital, Al-Taif, Saudi Arabia during the period from Jan. to Jun. 2012.

2. Materials and Methods

2.1. Bacterial Isolates

Fifty five strains of *S. aureus* were collected during the period from Jan. to Jun. 2012 from different departments of King Abdel Aziz Hospital, Al-Taif, Saudi Arabia. The strains were tested phenotypically and genotypically for the detection of MRSA. Thirty strains were collected from surgical wounds, 7 from sputum, 6 from blood, 5 from nasal swabs, 5 from skin and 2 from abdominal fluid. All isolates were identified according to colonial and microscopical morphology, catalase and coagulase production. The 55 strains were tested also by real-time PCR to identify MRSA isolates.

2.2. Culture Conditions and Media

The clinical isolates were first plated onto Columbia blood agar plates and incubated at 37˚C for 24 h. Identification of the MRSA strains was performed by standard procedures including colony morphology, catalase reaction and coagulase activity [8].

2.3. Antimicrobial Susceptibility Test

2.3.1. Detection of Oxacillin Resistance by Phenotypic Method

Characteristic colonies that were identified as *S. aureus* were tested for oxacillin resistance by using Mueller-Hinton agar and Disc-diffusion test according to NCCLS guidelines [9].

2.3.2. Antimicrobial Susceptibility Test to Other Drugs

Antimicrobial susceptibility test to a range of antimicrobial agents was performed using the following discs (augmentin, cotrimoxazole, fusidic acid, gentamicin, erythromycin, ciprofloxacin, clindamycin, amikacin, cepha lothin, linezolid, teicoplanin and vancomycin). The procedure was performed by adopting the Kirby-Bauer disc diffusion method using Muller-Hinton broth and agar and antibiotic discs according to NCCLS guidelines [9].

2.3.3. Detection of Oxacillin Resistance by Genotypic Method

2.3.3.1. DNA Extraction

DNA was extracted from different bacterial isolates using DNA extraction kit (Koma Bioteck Inc., Korea). Briefly, the bacterial pellet from each isolate was re-suspended in 200 µl lysozyme reaction solution (20 mg/ml lysozyme, 20 mM Tris-HCl pH 8, 2 mM EDTA, 1.2% triton) and incubated for 30 min at 37˚C. The sample was then incubated with proteinase K 20 µl and lysis buffer 200 µl at 60˚C for 30 min. and then further incubated at 95˚C for 15 min. Absolute ethyl alcohol 200 µl was then added to each sample, vortexed then loaded into XPTG mini column in a collection tube. The tubes were centrifuged at 13,000 rpm for 1 min. and the flow was discarded. The column was then washed twice then dried for 3 min at 13,000 rpm before the DNA was eluted in new clean sterile tube using 100 µl of sterile double distilled water.

2.3.3.2. Real-Time PCR

The real-time PCR assay was performed using comercial TaqMan hydrolysis probe based MERSA real time PCR detection kit (Liferiver, Shanghai, China) in Eppendorf Mastercycler® eprealplex. The detection of the amplified MERSA DNA amplicon was performed in fluorimeter channel FAM with the fluorescent quencher BHQ1. Amplification reactions were performed according to the in a volume of 25 µl containing 2.5 µl of DNA template, 21.5 µl reaction mix, 0.4 µl enzyme mix, 1 µl internal control according to the manufacturer's instructions. The cycling conditions included: 37˚C for 2 min, an initial denaturation step at 94˚C for 2 min followed by 40-cycles consisted of heating at 93˚C for 15 sec and annealing/elongation step at 60˚C for 1 min.

3. Results

Fifty five strains of *S. aureus* were collected during the period from Jan. to Jun. 2012 from different departments of King Abdel Aziz Hospital, Al-Taif, Saudi Arabia. Thirty strains were collected from surgical wounds, 7 from sputum, 6 from blood, 5 from nasal swabs, 5 from skin, and 2 from abdominal fluid as shown in (**Table 1**). The most frequent isolation of the *S. aureus* in relation to the age was noted in the age group of 21 - 40 years (40%), followed by those in the age group of 41 - 60

Table 1. Number of identified *S. aureus* and the percent of the resistant and susceptible of *S. aureus* to methicillin and oxacillin (by disc diffusion method).

Clinical specimens	No. of Isolated *S. aureus*	**MRSA**	**MSSA**
Skin	5	**4**	**1**
Nose	5	**3**	**2**
Blood	6	**4**	**2**
Wounds	30	**27**	**3**
Sputum	7	**6**	**1**
Abdominal fluid	2	**1**	**1**
Total	55	**45**	**10**

years (35%), >60 years (16%) and 0 - 20 years (9%) respectively (**Table 2**). A total number of 45 isolates (82%) were identified as MRSA while 10 isolates were MSSA. **Table 3** shows the antimicrobial resistance patterns of MRSA and MSSA strains. Glycopeptides and linezolid exhibited excellent activity against both MRSA and MSSA. The other antibiotics which were found to be effective against MRSA were amikacin (90.2%) and azitheromycin (78.2%). The overall drug resistance was more in MRSA isolates in comparison to MSSA. As many as 24% MRSA strains were resistant to multiple drugs in comparison to 10% of MSSA.

3.1. Detection of MRSA by Phenotypic Methods

Forty five strains (82%) out of 55 tested strains were resistant phenotypically to methicillin and oxacillin which was confirmed by real time PCR.

3.2. Evaluation of the PCR with Clinical Isolates

All of the 55 clinical isolates of MRSA were detected by real time PCR. The melting curve analysis showed a *Tm* of 60˚C (**Table 4** and **Figure 1**). All other clinical isolates of MSSA (10 strains) were tested negative by real time PCR.

4. Discussion

S. aureus is a major pathogen causing a wide spectrum of clinical manifestations and beta-lactam antibiotics are the drugs of therapeutic choice. Since the introduction of methicillin into clinical use on 1961, the occurrence of MRSA strains has enormously increased and MRSA is now one of the most important nosocomial pathogens worldwide [10]. Infections caused by MRSA require treatment with glycopeptide antibiotics, which are expensive and may have serious side effects. Therefore, rapid differentiation of MRSA strains from MSSA has important implications for the treatment but also for the management of patients with MSSA infections since extensive isolation measures is taken to limit the spread of MRSA [11]. Regarding the age of the patients, we found the occurrence of *S. aureus* to be higher among patients in the age group 21 - 40 years (**Table 2**). Mulla, *et al.*, [12] also reported that *S. aureus* was commonly isolated from patients in age group 21 - 30 years. MRSA has become prevalent with the discriminate and overuse of an-

Table 2. The frequency of *Staphylococcus aureus* isolation in relation to the age group.

Age group (years)	Number of isolation	Percentage
0 - 20	5	9
21 - 40	22	40
41 - 60	19	35
>60	9	16
Total	55	100

Table 3. Antimicrobial susceptibility patterns among (MRSA) and (MSSA) strains.

Antimicrobials	Percentage of susceptible MRSA isolates	Percentage of susceptible MSSA isolates
Ciprofloxacin	30	85
Gentamicin	36	68
amikacin	90.2	98.6
Azithromycin	78.2	82
Clindamycin	66.0	78.0
Cotrimoxazole	41.4	64.8
Fusidic acid	74	86
Erythromycin	44.6	59.4
augmentin	18.6	56.3
Cephalothin	14.0	62
Linezolid	100	100
Teicoplanin	100	100
Vancomycin	100	100

Table 4. Comparison of MRSA detection results for clinical isolates by culture identification and real time PCR.

Identification of Staphylococci	Number of isolates tested by PCR		
By conventional methods	Total	+ve	−e
S. aureus, Oxacillin resistant	45	45	0
S. aureus, Oxacillin susceptible	10	0	10
Total	55	45	10

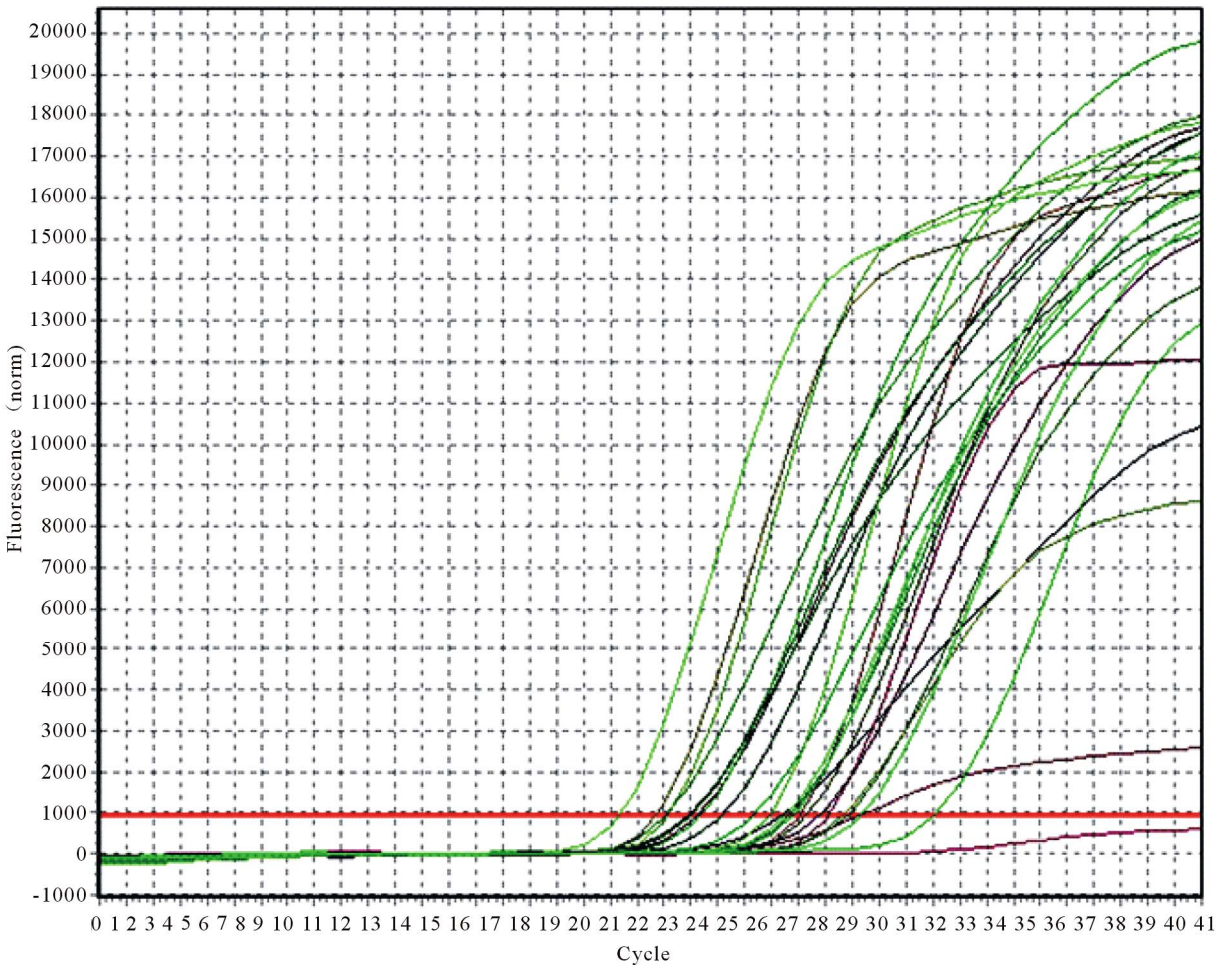

Figure 1. Real Time PCR results of some MRSA strains used in this study.

tibiotics. MRSA is of great concern not only because of its resistance to methicillin, but also because it is generally resistant to many other commonly used antimicrobials. Many studies have reported an increased in the incidence of MRSA during recent years [13]. Our study shows that MRSA was found to be 82% in consistent with other data [14]. In our study, it was noted that the sensitivity to most of antibiotics tested among MSSA was significantly higher than MRSA. In our study, amikacin sensitivity among the *S. aureus* strains was still high in comparison to the findings from other countries [15]. The sensitivity to amikacin was noted in 90.2% and 98.6% of MRSA and MSSA strains respectively. Our study showed that there was an increase in the rate of incidence of MRSA, which showed 100% susceptibility to vancomycin, linezolid and teicoplanin. Upon compareing our results of antibiotic susceptibility with other results inside Saudi Arabia, we found that all results (**Table 3**) in consistent with the results of Moussa and Hessan [15] except for ciprofloxacin in which our results showed only 30% of MRSA isolates were susceptible in comparison to 60% in their results.

MRSA strains are usually introduced into aninstitution by a colonized or infected patient. Rapid screening on admission of high-risk patients for MRSA is one of the control measures to limit the spread of MRSA strains. Several studies have found that such screening programs are cost-effective [16,17]. However, in the clinical laboratory, conventional culture methods are time-consuming and misinterpretation occurs sometimes when methicillin resistance is simulated by the hyper production of beta-lactamase [5] or coagulase activity is weak [18].

Based on these arguments, rapid and reliable methods for the detection of MRSA are required. The identification of MRSA strains based on the detection of the *mecA* gene and simultaneous detection of an *S. aureus* specific marker gene by molecular methods has been introduced as an interesting tool in clinical laboratories [7, 19,20]. In comparison to conventional PCR protocols with the detection of the PCR products on gel electrophoresis, real-time PCR assays are time-saving. All the presented assays, however, risk of co-amplifying the *S. aureus* specific marker gene together with the *mecA* gene from methicillin resistance coagulase negative staphylo-

cocci (MRCNS), leading to false-positive results. In clinical samples and consequently in enrichment broths MSSA and MRCNS could simultaneously be present.

In the present study, we reported the use and evaluation of a real-time PCR assay for MRSA screening from clinical isolates. The use of this new method together with a fast DNA extraction protocol to screen clinical isolates achieved a sensitivity of 100% (45 of 45 detected MRSA strains) and 100% specificity (no false-positive result). Results are available within 90 min and the total hands-on time was about 30 min.

The power of the PCR technique described here was confirmed by another study performed by Huletsky *et al.* [3]. Using five different forward primers in combination with three different probes and one reverse primer they were able to show that the vast majority of a world-wide collection of MRSA isolates was detected by their PCR assay. The sensitivity of their PCR strategy was 98.7% compared to 100% in the current study.

The real-time PCR assay used in the current study proved to be a sensitive and reliable tool for a rapid identification of MRSA from isolated colonies. The use of PCR technology is more expensive than the use of conventional methods. The clinical and economic benefit of saving time in regard to expenses remains to be elucidated. According to the hygiene policy in some countries, patients highly suspected to be colonized with MRSA are isolated in a single room until cultures become MRSA negative. An earlier MRSA negative report in such cases by a quick PCR assay as presented in this study will help to reduce isolation time and costs.

5. Conclusion and Recommendation

Our real-time PCR assay proved to be a sensitive andreliable tool for the rapid identification of MRSA fromisolated colonies. The use of PCR technology is more expensive than the use of conventional methods. The clinical and economic benefit of saving time in regard to expenses remains to be elucidated. According to the hygiene policy in some countries, patients highly suspected to be colonized with MRSA are isolated in a single room until cultures become MRSA negative. An earlier MRSA negative report in such cases by a quick PCR assay as presented in this study will help to reduce isolation time and costs.

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