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Full Length Research Paper

Detection of (*mecA*)gene in methicillin resistant *Staphylococcus aureus* (MRSA) at Prince A / Rhman Sidery Hospital, Al-Jouf, Saudi Arabia

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The study was conducted in A/ Sidery Hospital Al-Jouf province, Saudi Arabia during, the period September 2008 to May 2009. A total of 930 patients attended the Hospital for routine investigations were screened for urinary tract infection. A total of nine hundred and thirty urine specimens were received in the laboratory for culture, 640 specimens showed significant growth from which 100 *Staphylococcus aureus* species were recovered. Disk diffusion method and BD phoenix TM system antibiotic susceptibility were used, 15 methicillin resistant *S. aureus* isolates were identified, polymerase chain reaction (PCR) was used to amplify both the *S. aureus* specific sequence gene and *mec*A gene with the amplicon size of 107 and 532 bp. All the isolates (n=100) expressed *S. aureus* specific sequence gene in their PCR products. The results of the PCR revealed 13/15 isolates demonstrating both resistance to methicillin and expression of *mec*A gene, while the remaining two showed the resistance to methicillin by the disc diffusion method without the expression of *mec*A gene. All the isolates (n = 100) were sensitive to vancomycin.

Key words: *mecA*, MRSA, methicillin resistance polymerase chain reaction (PCR), Staphylococcus *aureus*, Al-Jouf, Saudi Arabia.

INTRODUCTION

Staphylococcus aureus is one of the most frequent bacterial pathogens in humans. It causes skin infections, osteoarthritis and respiratory tract infections in the community, as well as postoperative and catheter-related infections in hospitals (Didier et al., 2004) Methicillin-resistant *S. aureus* (MRSA) has become a major public health problem worldwide (Jarvis et al., 2007). The burden of MRSA continues to rise, with a growth rate of 14% of all *S. aureus* strains from clinically significant samples in New South Wales, Australia (Nimmo et al., 2006). The rising colonization rates lead to the increasing of infection rates in the community and in hospitals. The consequence to the health care system is longer hospital stays and greater costs, which approximately double the expenditure per patient (Kim et al., 2001*).* The patient risks include significantly higher mortality and morbidity rates with invasive MRSA infection (Lodise and McKinnon, 2005, Kearns et al., 1999) Within U.S. hospitals, nearly 60% of nosocomial *S. aureus* infections acquired in intensive care units are methicillin resistant (NNIS, 2004). Health care workers may carry MRSA on their hands or clothes following their contact either with to asymptomatic carriers or patients who have clinical infection. Health workers may then, unknowingly transmit the organism to other patients. The contaminated environmental surfaces also contribute to the MRSA transmission. Thus, symptomatic patients constitute a small portion of the actual reservoir of MRSA within hospitals resulting in an iceberg phenomenon (Harbarth et al., 2000). The world wide emergence of community acquired methicillin resistant *S. aureus* (CA-MRSA) can have severe public health implications (Calfee et al., 2003). The differentiation between community-acquired MRSA and hospital acquired MRSA (HA-MRSA) is becoming difficult to understand, since CA-MRSA could spread into hospitals (Wannet et al., 2004). The risk of

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the acquiring MRSA in the hospitals increased by severity of illness (Ibelings and Bruining, 1998), length of stay, (Law and Gill., 1998), use of intravascular devices (Pujol et al., 1994) and the intensity of exposure to infected patients (Merrer et al., 2000). Infection control measures include screening, (Girou et al., 1998) and (Lucet et al., 2003) segregation of positive patients, (Arnoldet et al., 2002), eradication of carriage (Hill et al., 1988) and good standards of general hygiene (Rampling et al., 2001).

MATERIALS AND METHODS

The study was conducted in A/ Sidery Hospital Al-Jouf province, Saudi Arabia, during the period September 2008 to May 2009. A total of 930 patients attended the hospital for routine investigation which was screened for urinary tract infection, preliminary identification of the isolates was performed on the basis of colonial morphology, cultural characteristics on agar media, gram's staining reaction and biochemical reaction results using standard methods (Kloos and Bannerman, 1999). *S. aureus* species were identified and methicillin resistant *S. aureus* (MRSA) isolates were detected at the time of initial culture using the disk diffusion method. Antibiotic sensitivity of the isolates initially demonstrating resistance to methicillin was confirmed using BD phoenix TM (System, Becton, Dickinson Company, Shannon, Ireland) according to the recommendations given by the national reference centre in Saudi Arabia. The sensitivity pattern of the isolates was tested for the antibiotics listed in Table 1. Wizard R genomic DNA purification kit+ was used according to manufacturer's instructions (Promega) to isolate the DNA from *S. aureus* clinical isolates.

Amplification of *S. aureus* specific sequence gene and *mecA* gene

PCR was used to amplify both the *S. aureus* specific sequence gene and *mecA* gene with the amplicon size of 107 and 532 bp using primers described by Martineau et al. (1998). The 3-end region of the *S. aureus* specific gene was amplified using A 30 nucleotide forward primer 5'- AAT CTT TGT CGG TAC ACG ATA TTC TTC ACG -3' and A30 nucleotide reverse primer, 5'-CGT AAT GAG ATT TCA GTA GAT AAT ACA ACA-3' (which hybridize to sites 5-34 and (112-83), respectively, (Martineau et al. 1998). While The 3-end region of the *mecA* gene was amplified using A 22 nucleotide forward primer 5'- AAA ATC GAT GGT AAA GGT TGG C - 3' and A22 nucleotide reverse primer, 5'- AGT TCT GCA GTA CCG GAT TTG C-3' (which hybridize to sites 1282-1301 and 1814-1793) (Robert Koch institute, 2003). The PCR reaction mixture for the detection of *mecA* gene and *S. aureus* specific gene consisted of 1 ml of sample containing template DNA. 1.5 u of tag DNA polymerase, 10 µl of 10 x PCR amplification buffer 10 pmol each primer, 200 m/mole deoxynucleotide triphosphate (dNTPS) and distilled water to a final volume of 5010 µl. A total of 40 cycles were used to amplify 532 bp of *mecA* gene and 107 bp of *S. aureus* specific gene. DNA denaturation occur at 94°C for 30 sec primers annealing at 55°C for 30 sec extension of the two strands at 72°C for 60 s and a final extension step of 4 mins. The PCR products were analyzed on a 1.5% agarose gel five micro liters of the PCR products were loaded into 1.5% phorecus agarose (Biogene, UK) and electrophoresis was performed in .5x TBE buffer at 180 V for 3 h. The gels were subsequently stained with 1 µg/ml ethidiumbromide (Sigma, U.K) for 30 min, visualized under UV and photographed.

RESULTS

Nine hundred and thirty urine specimens were received in the laboratory for culture, 640 specimens showed significant growth from which 100 *S. aureus* species were recovered. Using disk diffusion method and BD phoenix TM system antibiotic susceptibility testing identified 15 methicillin resistant *S. aureus* isolates. The antimicrobial agents used their abbreviations potency and origin is shown in Table 1.

All the isolates (n = 100) expressed *S. aureus* specific sequence gene in their PCR products, which confirmed the assumption that all the strains were *S. aureus*. The result of the PCR revealed 13/15 isolates demonstrating both resistance to methicillin and expression of *mecA* gene, while the remaining two showed the resistance to methicillin by the disc diffusion method without the expression of *mecA* gene. All the isolates (n=100) were sensitive to vancomycin.

In Figure 1, all the *S. aureus* isolates were sensitive to vancomycin, while only 2% were sensitive to penicillin and 15% were identified as MRSA. Meropeneme and amoxicillin expressed relatively high activity against the isolates (80 and 74%). Cephalosporin and gentamicin showed the same activity and the least active antibiotic was ciprofloxacin.

According to the Figure 2, all MRSA isolates were sensitive to vancomycin and resistant to penicillin. The isolates expressed high resistant against cephalosporin, gentamicin and ciprofloxacin and relatively low resistant against meropeneme and amoxicillin.

In Figure 3, a 100 molecular weight marker was applied at the first and last well of the gel to identify the isolated genes. A negative control (methicillin susceptible *S. aureus*) PCR product was applied next to the molecular weight marker. (No band on the figer). PCR product of *S. aureus* specific gene 107 bp was applied on lane 3, 5, 7, 9 and 11 which showed a clear band confirmed that, all the isolates were *S. aureus*. PCR product of *mecA* gene 532 bp for the same isolates was applied on lane 4, 6, 8, 10 and 12 which showed clear bands confirmed that, all the isolates were MRSA.

DISCUSSION

Detection of MRSA is important for patient care and appropriate utilization of infection control resources. Methicillin-resistant *S. aureus* (MRSA) is a significant pathogen that has emerged over the last four decades, causing both nosocomial and community-acquired infections. Rapid and accurate detection of methicillin resistance in *S. aureus* is important for the use of appropriate antimicrobial therapy and for the control of nosocomial spread of MRSA strains. We evaluated the efficiency of the disk diffusion method, BD phoenix TM

Table 1. Interpretation of the inhibition zone diameter data.

Antibiotic	Disk potency	Resistant	Sensitive
Penicillin (P)	10 units	<28	> 29
Methicillin (ME)	5 µg	<9	> 14
Gentamycin (Gen)	10 µg	<12	> 15
Ciprofloxacin (CIP)	5 µg	<18	> 21
Meropenem (Mer)	10 µg	<13	> 16
Amoxycillin/Clavulanic Acid (AMX)	30 µg	<17	> 21
Vancomycin (Van)	30 µg	<17	> 21

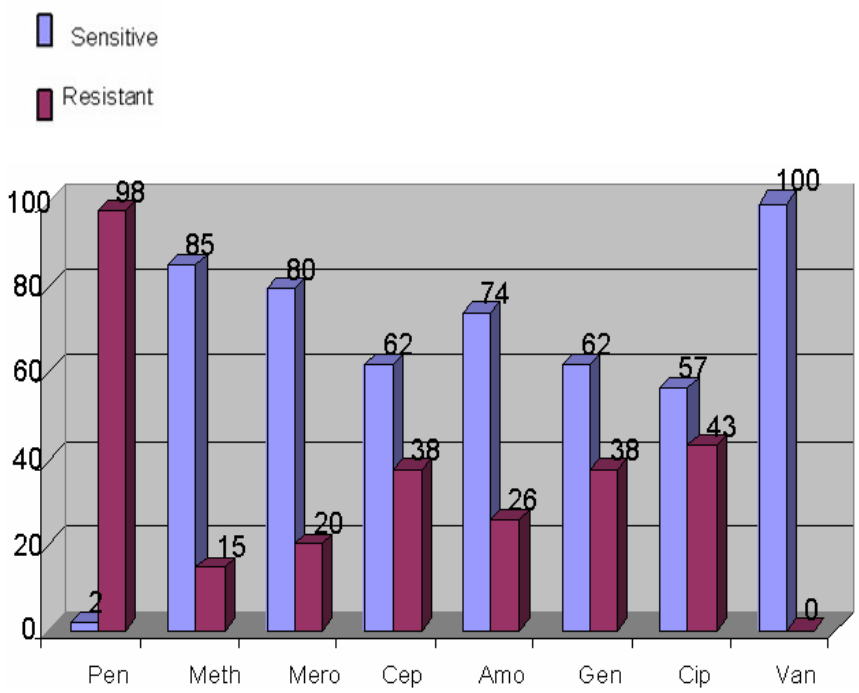

Figure 1. Susceptibility of *S.aureus* isolates to different antibiotics.

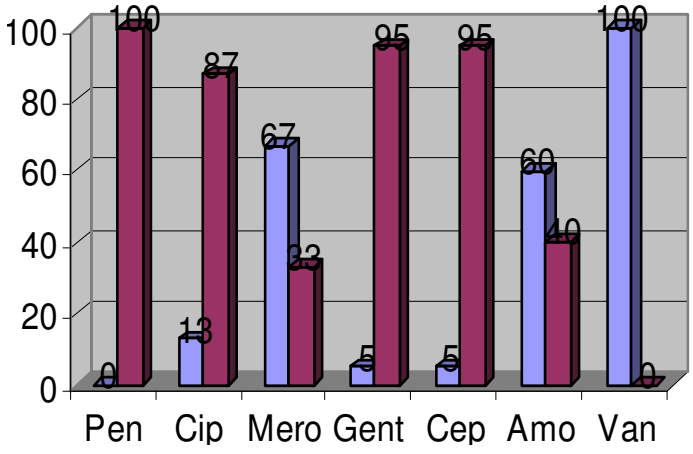

Figure 2. Susceptibility of MRSA isolates to different antibiotics.

system and PCR for detection of methicillin resistance in 100 isolates of *S. aureus*, 13 mecA positive and 87 mecA negative. The PCR of mecA gene was used as the gold standard for the evaluation of the other two methods. The percentages of sensitivity and specificity were as follows; disk diffusion 97 and 100%, and BD phoenix TM system 100 and 100%. The two methods presented high sensitivity and specificity, but BD phoenix had the advantage of giving a reliable result, equivalent to PCR. The incidence of urinary tract infection with *S. aureus* was found to be 15.6%; Beta lactamase producers within the *S. aureus* isolates were 86%, this result agreed with Fukatsu et al. (1990) who reported that, 81.3% of *S. aureus* were beta lactamase producers in Japan. Depending on the methicillin susceptibility testing result15 (15%) of the *S. aureus* isolates were classified as MRSA,

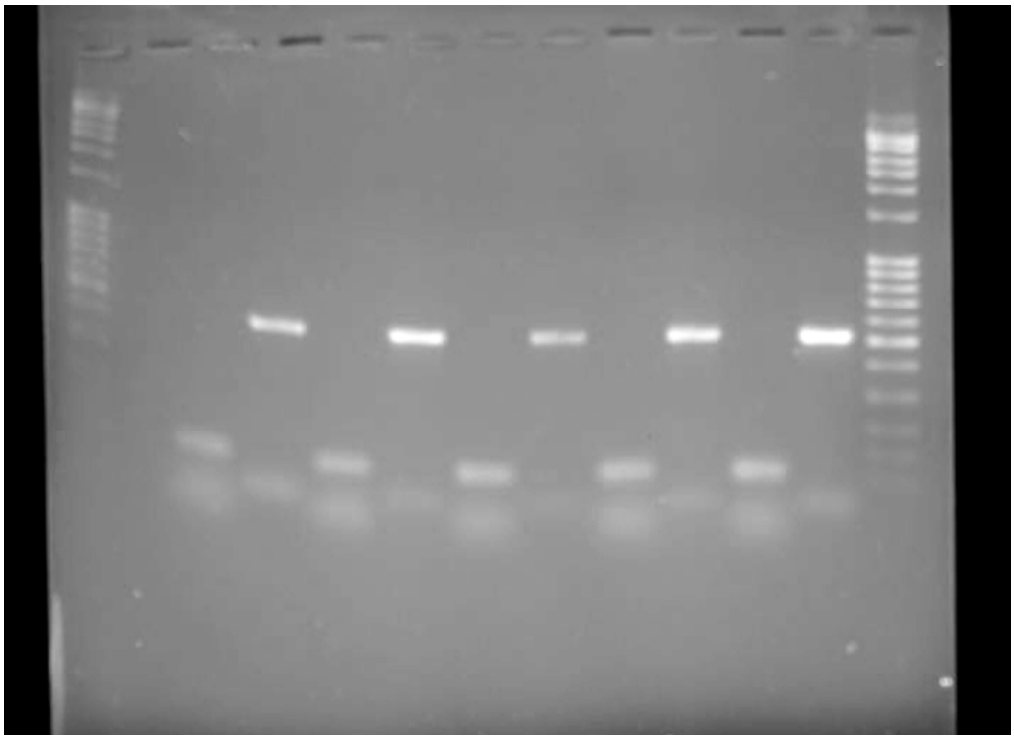

Figure 3. A 100 molecular weight marker applied at the first and last well of the gel to identify the isolated genes. A negative control (methicillin susceptible *S. aureus*) PCR product was applied next to the molecular weight marker. (No band on the figure). PCR product of *S. aureus* specific gene 107 bp was applied on lane 3, 5, 7, 9 and 11 which showed a clear band confirmed that, all the isolates were *S. aureus*. PCR product of *mecA* gene 532 bp for the same isolates was applied on lane 4, 6, 8, 10 and 12 which showed clear bands confirmed that, all the isolates were MRSA.

13/15 (8.7%) of the MRSA isolates expressed *mecA* gene by PCR typing in addition to beta lactamase enzyme production, this result agreed with Tenover et al. (1994) who stated that, the resistance in *S. aureus* mainly involve two mechanisms the expression of beta lactamase and *mecA* gene. In this study, the MRSA resistance pattern was studied against the ten antibiotics excluding penicillin and vancomycin, twelve isolates proved to be resistant to the ten antibiotics, five to eight, three to six and three to four antibiotics. Regarding to the disc diffusion method for the detection of MRSA busy laboratories processing, screening and reading of the specimen results are time-consuming, all isolates are confirmed with tube coagulase and susceptibility testing and keeping agar plates for an extended period (48 h) increases the workup of suspicious colonies significantly, with a small increase in MRSA detection this agreed with Diederen et al. (2006) and Diederen et al. (2005).

In conclusion, molecular techniques remains the most sensitive method in detecting *S. aureus* at both genus and species level and with 100% accuracy in detecting MRSA, when compared with the classical identification method and this agreed with Martineau et al. (2001). In addition, for greater detection rates, molecular methods have the shortest turn around time. Although, molecular testing remains expensive relative to conventional agar-based detection, there is an overall cost savings, especially if molecular testing is directed at high-risk populations.

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