Molecular detection of methicillin resistant \textit{Staphylococcus aureus} (MRSA) and methicillin resistant coagulase-negative \textit{Staphylococcus} (CoNS) in Iran

Neda Razavi Davoodi\textsuperscript{1}, Jalil Vand Yousefi\textsuperscript{1}, Naser Harzandi\textsuperscript{1}, Ali Hajrafi\textsuperscript{1}, Bahareh Rajaei\textsuperscript{2}, Siyavosh Gerayesh-Nejad\textsuperscript{4}, Mohammad Reza Aghasadeghi\textsuperscript{2}, Arfa Moshiri\textsuperscript{5}, Ahmad Reza Bahremand\textsuperscript{6} and Seyed Davar Siadat\textsuperscript{1,2,3,*}

\textsuperscript{1}Department of Microbiology, Karaj Branch, Islamic Azad University, Karaj, Iran. \\
\textsuperscript{2}Department of Hepatitis and AIDS, Pasteur Institute of Iran, Tehran, Iran. \\
\textsuperscript{3}Department of Microbiology, Pasteur Institute of Iran, Tehran, Iran. \\
\textsuperscript{4}Department of Biochemistry, Tehran University of Medicine Sciences, Tehran, Iran. \\
\textsuperscript{5}Department of Biotechnology, School of Allied Sciences, Tehran University of Medical Sciences, Tehran, Iran. \\
\textsuperscript{6}Department of Mycobacteriology, Pasteur Institute of Iran, Tehran, Iran. \\

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The incidence of methicillin resistance has risen among nosocomial isolates of \textit{Staphylococcus aureus} and coagulase-negative staphylococci. The present study was carried out to investigate the prevalence of methicillin-resistant \textit{Staphylococcus aureus} (MRSA) and methicillin-resistant coagulase negative staphylococci (MRCoNS) and to determine their antibiotic susceptibility pattern. A total of one hundred and thirty clinical staphylococcal isolates recovered from blood, tracheal aspirate, urine and wound specimens were evaluated for susceptibility to penicillin, amikacin, ciprofloxacin, vancomycin, erythromycin, ceftriaxone, methicillin, rifampin and gentamicin by Disk diffusion method and molecular detection of \textit{mecA} gene. The results showed that MRCoNS were more resistant to these antibiotics as compared to MRSA and the most effective antibiotic to use for staphylococcal isolates is vancomycin showing (100\% of \textit{S. aureus} and 90\% of CoNS) efficacy. The \textit{mecA} gene was detected in 56\% of the isolated \textit{S. aureus} and 70\% of the CONS isolates. The prevalence of methicillin resistant staphylococci in Iran was very high and 45.4\% of MRSA and MRCoNS isolates were at least resistance to 3 or more classes of antibiotics. The high prevalence of MRSA immerge in Iran could be originated due to antibiotic pressure and poor control measures on the application of antibiotics such as methicillin.

Key words: \textit{Staphylococcus aureus} (\textit{S. aureus}), coagulase-negative staphylococci (CoNS), \textit{mecA} gene, methicillin-resistance.

INTRODUCTION

\textit{Staphylococcus aureus} is an important nosocomial pathogen distributed widely across the world (Zhang and Sparling, 2004). They are also one of the most important common colonizer of the human skin. It is the casual atal agent of most staphylococcal infections. Staph-related illnesses ranging from mild to severe and potentially diseases, causing surgical wound infections, urinary tract infections, bloodstream infections (sepsis) and pneumonia (Amghalia and AL-Haj, 2009).

Coagulase-negative staphylococci (CoNS) were regarded as part of the normal skin flora prior to the 1970s; but,
they are now recognized as important causes of human infections and considered as major nosocomial pathogens (Al-Talib et al., 2009). On the other hand, since CoNS are usually resistant to multiple antibiotics, often serve as reservoirs of antimicrobial resistance determinants. Therefore, it is important to distinguish between S. aureus strains and CoNS (Zhang and Sparling, 2004). Treatment of staphylococcal infections was revolutionized in the 1940s by the introduction of the antibiotic penicillin. In the year 1960, Methicillin, the first semisynthetic penicillin was developed against penicillin-resistant staphylococci. However, methicillin-resistant S. aureus (MRSA) strains were identified, within the year of its introduction (Shanthi and Sekar, 2009). Resistance to methicillin is mediated by the acquisition of penicillin binding protein PBP-2a encoded by the meca gene, which exhibits a low affinity for β-lactam antibiotics (Wielders and Fluit, 2002).

Many methicillin-resistant staphylococci have a high prevalence of multidrug resistance leading to an overall increase in the incidence of nosocomial staphylococcal infections (Petinaki and Kontos, 2001). During the past decade, increasing rates of antibiotic resistance among S. aureus strains and CoNS caused a major health concern and have been considered a global crisis. Therefore, it is necessary to study the ways to control this bacterium and their ability to counteract antibiotic effects (Pérez-Roth et al., 2001). The aim of this study was to evaluate the antibiotic resistance profile among clinical isolates of Staphylococcus spp. with respect to the presence of the meca gene in these isolates.

MATERIALS AND METHODS

Bacterial isolates

A total of 130 Staphylococcal species isolated from various clinical cases during the years 2009 to 2010 were analyzed in the present study. The origins of these isolates were indicated in Table 1.

Identification of staphylococcal isolates

The bacterial isolates were cultured on blood agar plates with 5% sheep blood and incubated aerobically for 24 h at 37°C. The isolates of S. aureus and CoNS were identified by Gram staining, Catalase test, Mannitol fermentation and D’nase test by standard laboratory procedures as described by Brown and Edwards (2005).

Table 1. Origin of Staphylococcus spp.

<table>
<thead>
<tr>
<th>Origin of sample</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracheal</td>
<td>15</td>
</tr>
<tr>
<td>Urine</td>
<td>28</td>
</tr>
<tr>
<td>Wound</td>
<td>27</td>
</tr>
<tr>
<td>Blood</td>
<td>60</td>
</tr>
<tr>
<td>Total</td>
<td>130</td>
</tr>
</tbody>
</table>

The coagulase test was done to discriminate between S. aureus and CoNS.

Antimicrobial susceptibility test

The antimicrobial susceptibility test was performed for each of the 130 staphylococcal isolates using the standard disk diffusion method on Muller-Hinton agar. Disks prepared by MAST company (Mast Co, Merseyside, UK) were used to determine the susceptibility of isolates to penicillin (10 µg), meticillin (5 µg), erythromycin (15 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ceftriaxone (30 µg), vancomycin (30 µg), rifampicin (5 µg), and amikacin (30 µg). The results were determined according to the Clinical and Laboratory Standards Institute (CLSI) recommendations (CLSI, 2009). S. aureus (ATCC 25923) and S. epidermidis (ATCC 14990) were used as quality control strains.

Genomic DNA extraction

The isolated colonies from blood agar were inoculated into LB broth and incubated at 37°C for 18 h. Bacterial lysates for PCR were prepared by centrifuging the 600 µl culture at 15,700 ×g for 2 min; the supernatant was removed and the pellets were resuspended in 200 µl of lysis solution (25 mM Tris HCl, 10 mM EDTA, twenty units of lysostaphine [pH 8.0]) and the suspension was incubated at 37°C for 30 min. DNA extraction was performed by Phenol-Chloroform extraction method as described by Sambrook et al. (1989).

meca PCR assay

All the 130 staphylococcal isolates were analyzed by PCR for the detection of meca gene responsible for the methicillin resistance, via PCR assay as described by Al-Talib et al. (2009). PCR was performed with primers 5’-ACGAGTAGATGCTCAATATAA-3’ as forward and 5’- CTTAGTCTCTTTAGCGATTGC -3’ as reverse. The PCR reaction mixture consisted of 2 µl DNA template, 5 U Tag DNA polymerase, 3mM MgCl2, 1 × PCR amplification buffer, 10 pmol each primer and 200 µM deoxynucleotide triphosphate (dNTPS). A total of 30 cycles were used to amplify the 293 bp meca gene. The PCR was performed on the thermocycler (Eppendorf Mastercycler® MA) with one cycle of initial denaturation at 94°C for 3 min, 30 cycles of denaturation at 94°C for 30 s, annealing for 30 s at 60°C, and extension at 72°C for 30 s, followed by an extra cycle of annealing at 60°C for 30 s, and a final extension at 72°C for 5 min. The PCR products were stained with ethidium bromide and visualized on 1.5% agarose gel with a UV light transilluminator. Control marker with molecular mass of 100bp was used (fermentase, Lithuania). The positive and negative control strains used for the meca gene detection were S. aureus (ATCC 33591) and S. aureus ATCC25923, respectively.
DNA sequencing and submission

The 293 bp PCR product attributed to the mecA gene was sequenced using the ABI Capillary System (SEQLAB, Berlin, Germany). Sequence was analyzed using online BLAST software (http://www.ncbi.nlm.nih.gov/BLAST) and submitted to the EMBL/GenBank database (www.ncbi.nlm.nih.gov).

Statistics

The results were analyzed with SPSS16 by chi-square test. P value of < 0.05 was considered significant.

RESULTS

Prevalence of staphylococcal species

Out of the 130 isolates, 100 were S. aureus and 30 were CoNS (76.9 and 23% respectively) isolates. S. aureus recovered from wound (n = 27), blood (n = 30), tracheal aspirate (n = 15), urine (n = 28) and all CoNS were isolated from blood of patients (Table 1). Of 130 patients, 55 S. aureus and 9 CoNS isolates were related to female and 45 S. aureus and 21 CoNS isolates were recovered from male.

Antimicrobial susceptibility test

Of the 130 isolates, only 4 (3% CoNS) of the isolates were sensitive to the all of the tested antimicrobial agents and all S. aureus isolates were sensitive to vancomycin. However, 90% of the CoNS isolates were sensitive to vancomycin %. Rifampin was the second most effective antibiotic and only 1 and 16.6% of S. aureus and CoNS isolates were resistant to rifampin respectively. Among antibiotics used in this study, penicillin showed the least anti-staphylococcal activity for example, over 80% of CoNS and 100% of S. aureus isolates were resistant to this antimicrobial agent.

The antimicrobial resistance profiles among S. aureus and CoNS isolates were as follow: 100%, 80% of isolates were resistant to penicillin, 58%, 73.3% to meticillin, 44%, 66.6% to erythromycin, 60%, 56.6% to gentamicin, 56%, 60% to ciprofloxacin, 42%, 50% to ceftriaxone, 0%, 10% to vancomycin, 16%, 16.6% to rifampin, 53%, 66.6% to amikacin, respectively (Table 2).

Multidrug resistance was reported as a single isolate resistant (intermediate or complete) to 3 or more unique antimicrobial classes. Of the 130 isolates, 59 isolates (45.4%) were considered as MDR Staphylococcal isolates (Table 3).

The rate of antibiotic resistant isolates of MRSA and MRCNS were shown in Table 4. The highest resistance of MRSA and MRCNS isolates related to gentamicin and amikacin/erythromycin with 71 and 85.7% resistance frequency respectively.

The prevalence of MRSA was 58% among all studied isolates but was highest among S. aureus isolated from blood (75%) and was lowest among isolates related to wound infections (29%). The prevalence of methicillin resistance was 42 and 30% among tracheal aspirate and urine infection isolates respectively (Table 3).

PCR assay

The mecA gene was detected in all 130 isolates of staphylococci. 56 isolates (56%) of 100 S.aureus isolates and 21 isolates (70%) of 30 CONS isolates were mecA positive. The 293bp PCR product of mecA gene is shown in Figure 1.

Nucleotide sequence and accession number

The 293 bp PCR product related to mecA gene was sequenced. The comparison between sequence result and sequences in the Gene Bank database revealed high identity to the sequence of mecA with the GenBank accession no. JN258594.

Statistics

Of the 30 CoNS isolates, 7 (77.7%) and 18 (85%) were found to be MRCoNS isolates recovered from women and men respectively. Of the 100 S. aureus isolates recovered from women and men 37 (67.2%) and 33 (73.3%) were found to be MRSA isolates respectively. On the basis of sex, the rate of MRSA and MRCNS strains among men and women were (p = 0.43 )and (p = 0.28) respectively.

DISCUSSION

During the last decade, S. aureus and CoNS have emerged as important nosocomial pathogens and rising antibiotic resistance in these organisms is a major public health concern (Diekema and Pfaller, 2001). Recognition and discrimination of S. aureus and CoNS and detection of methicillin resistance are essential for prompting effective antimicrobial therapy and for limiting the unnecessary use of certain antibiotic’s classes (Zhang and Sparling, 2004).

Staphylococcal isolates were tested against a panel of antimicrobials along with penicillin and erythromycin, amikacin, ceftriaxon, methicillin, ciprofloxacin, vancomycin, rifampin and gentamicin (Table 2). In our study it is showed that S. aureus and CoNS isolates were most resistant to penicillin with the resistance frequency of 100%, 80% respectively. 29% of S. aureus showed sensitivitiy to gentamicin. Furthermore in many studies, high resistance rate against erythromycin, gentamicin in S. aureus were reported (Shanthi and Sekar, 2009;
Table 2. Antibiotic resistance profile of S. aureus and coagulase negative staphylococci isolates.

<table>
<thead>
<tr>
<th>Organism (no.)</th>
<th>Antibiotic resistance¹, no. (%)</th>
<th>MDR²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>MET</td>
</tr>
<tr>
<td>S. aureus (100)</td>
<td>100(100)</td>
<td>58(58)</td>
</tr>
<tr>
<td>CoNS (30)</td>
<td>24(80)</td>
<td>21(73.3)</td>
</tr>
<tr>
<td>Total (130)</td>
<td>124(95.4)</td>
<td>79(60.8)</td>
</tr>
</tbody>
</table>

¹Abbreviation of mentioned antibiotics are P, Penicillin; MET, Methicillin; V, Vancomycin; CIP, Ciprofloxacin; CTX, Ceftriaxone; GEM, Gentamicin; ERY, Erythromycin; AN, Amikacin; RIP, Rifampin.

²Multidrug resistance was reported as a single isolate resistant (intermediate or complete) to 3 or more unique antimicrobial classes.

Table 3. Antimicrobial drug resistance among different clinical isolates of Staphylococcus aureus isolates.

<table>
<thead>
<tr>
<th>Origin of the isolate</th>
<th>Antimicrobial drug resistance¹, no. (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>MET</td>
</tr>
<tr>
<td>Tracheal</td>
<td>15(100)</td>
<td>6(42)</td>
</tr>
<tr>
<td>Urine</td>
<td>28(100)</td>
<td>24(30)</td>
</tr>
<tr>
<td>Wound</td>
<td>27(100)</td>
<td>20(29)</td>
</tr>
<tr>
<td>Blood</td>
<td>30(100)</td>
<td>8(15)</td>
</tr>
<tr>
<td>Total</td>
<td>100(100)</td>
<td>58(58)</td>
</tr>
</tbody>
</table>

¹Abbreviation of mentioned antibiotics are P, Penicillin; MET, Methicillin; V, Vancomycin; CIP, Ciprofloxacin; CTX, Ceftriaxone; GEM, Gentamicin; ERY, Erythromycin; AN, Amikacin; RIP, Rifampin

Table 4. Antimicrobial drug resistance in mecA positive and mecA negative Staphylococcus spp.

<table>
<thead>
<tr>
<th>Organism (mecA)</th>
<th>Antibiotic resistance¹, no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
</tr>
<tr>
<td>S. aureus (mecA positive)</td>
<td>100(100)</td>
</tr>
<tr>
<td>S. aureus (mecA negative)</td>
<td>100(100)</td>
</tr>
<tr>
<td>CoNS (mecA positive)</td>
<td>14(66.6)</td>
</tr>
<tr>
<td>CoNS (mecA negative)</td>
<td>10(47.6)</td>
</tr>
</tbody>
</table>

¹Abbreviation of mentioned antibiotics are P, Penicillin; V, Vancomycin; CIP, Ciprofloxacin; CTX, Ceftriaxone; GEM, Gentamicin; ERY, Erythromycin; AN, Amikacin; RIP, Rifampin

Khadri and Alzohairy, 2010). Resistance rate to ciprofloxacin, 56% of S. aureus and 60% of CoNS, obtained in this study was similar to the 51% resistance obtained by Mohan and Jindal, (2002) in clinical CoNS isolates but was much lower than that obtained by Rao and Prabhakar, (2011), who observed 88.27% resistance in S. aureus isolates. These studies showed that the staphylococcal isolates were often resistant to multiple antibiotics, leading to much more difficulty in treatment. This may be due to the counter availability of antibiotics without prescription and
countries were as follow: in Turkey 44% of isolates (Sareyyupoglu et al., 2008), in Denmark 51% (Poulsen et al., 2003) and in China 60% (Yao and Yu, 2010). In fact, the prevalence of mecA positive S. aureus isolates was reported to be at the range of 43 to 65% in these studies. The frequency of mecA gene among CoNS isolates observed in this study was higher than the results obtained by Monsen et al. (2002) and Sareyyupoglu et al. (2008) which were reported to be 44 and 56%. This high prevalence of MRCoNS isolates in Iran may be related to the inappropriate use of methicillin and insufficient infection control measures in hospitals.

Comparative study between methicillin disk diffusion method and PCR results revealed that out of 77 isolates (56 S. aureus and 21 CoNS isolates) that were positive for the mecA gene, two and one isolates yielded false positive results with methicillin disk respectively. This finding has already been reported by Cekovska and Panovski (2005). Our results confirmed that PCR is one of the most important molecular tools available for identification of methicillin-resistant staphylococci. With respect to high emergence of MRSA and methicillin-resistant CoNS infections occurring on a worldwide basis, identification and treatment of these organisms in hospital are important, helping to avoid morbidity and spread of infections (Bashir and Mujahid, 2007).

Conclusion

This study indicated that the prevalence of MRSA and methicillin-resistant CoNS isolates is rising and the pattern of antibiotic susceptibility to first line antibiotics is changing. Vancomycin and rifampin are suggested to be effective antibiotics against methicillin-resistant staphylococci.

REFERENCES


