The prevalence of methicillin resistant Staphylococcus aureus (MRSA) isolates with high-level mupirocin resistance from patients and personnel in a burn center

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ABSTRACT

The rate of the MRSA strains, particularly at burn centers, is increasing worldwide. Detection of mupirocin resistance MRSA strains in the burn centers particularly from personnel will help to control these strains. For this purpose, a total of 116 Staphylococcus aureus isolates from the patients (burns) and personnel (nostrils) in Ahvaz Taleghani hospital (Iran) were investigated. The methicillin and mupirocin resistant isolates were detected by multiplex amplification of the mecA and iles-2 genes. The mecA was found among 80% of isolates. The rates of mupirocin resistant strains among personnel and patients were 70% and 6%, respectively. The carriage rates of the S. aureus, MRSA and MRSA with high-level mupirocin resistance in the personnel were 40%, 34% and 28%, respectively. In conclusions, the high prevalence of MRSA strains in the patients showed the potential outbreak of the MRSA in the burn center and highlighted the need of antibiotic susceptibility monitoring of MRSA. Moreover being personnel as a main reservoir in terms of MRSA strains with high-level mupirocin resistance emphasizes the screening of the personnel in terms of the MRSA in the healthcare system especially in the burn center.

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1. Introduction

Despite applying surveillance program in the hospitals, rate of infection and colonization with methicillin-resistant Staphylococcus aureus (MRSA) and subsequently healthcare costs are increasing [1–3]. The major concern about this organism is the serious infections caused by multi-drug resistant strains [2,4,5]. The sequels include prolonged hospitalization, economic burden, bacteremia or sepsis and even death [6]. MRSA is one of the predominant bacteria that causes wound infections in burn patients and its outbreak in the burn units is common. Many studies have concerned about the worldwide increase of this organism and high potency of mortality and morbidity in the burn patients [4,7–11]. The only approved antibiotic for topical use against MRSA is mupirocin (pseudomonic acid A). Unfortunately, the efficacy of mupirocin for such usage remains controversial [1].
2. Materials and methods

2.1. Collection of clinical isolates

From June 2007 to December 2008, a total of 116 S. aureus isolates were collected from personnel and burn patients who were admitted to Ahvaz Taleghani hospital. Specimens were collected by swabbing of both nostrils of the personnel (n = 50) and subsequently cultured on the sheep blood agar and mannitol salt agar media. Isolates from patients (n = 117) were cultured from burns. Duplicate isolates from the same patient were not included in the study. Standard biochemical tests such as Gram staining, production of catalase, coagulase, DNase and fermentation of mannitol were used for the identification of S. aureus [17].

2.2. Multiplex polymerase chain reaction (MPCR)

Extraction of DNA from isolates and PCR amplification was done according to the previous study [18]. DNA was extracted using boiling method. Primers FemB1 (5'-TTA CAG AGT TAA CTG TTA CC-3) and FemB2 (5'-ATA CAA ATC CAG CAC GCT CT-3), MecA1 (5'-GTA GAA ATG ACT GAA GTG CCG ATA A-3) and MecA2 (5'-CCA ATT CCA CAT TGT TTC CCA A-3), and MupA (5'-ATT ATT CTA AAG CTA AAG GGT C-3) and MupB (5'-AA TAA ATC AGC TGG AAA GTG TTT-3) were used for detection of S. aureus, mecA and ileS-2 genes, respectively. The PCR reaction and condition were similar to the method explained by Perez-Roth et al. [28]. The PCR products (10 μl) were run on 1.5% agarose gel and stained with ethidium bromide after electrophoresis in 0.5× TBE for 100 min at 100 mV.

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility of S. aureus isolates was performed on the isolates that were collected from patients according to the Clinical and Laboratory Standards Institute (CLSI) guidelines by disk diffusion method [19]. The following antibiotics were used: penicillin (10 units), oxacillin (1 μg), vancomycin (30 μg), teicoplanin (30 μg), amikacin (30 μg), gentamicin (10 μg), mupirocin (5 μg), linezolid (μg), ciprofloxacin (5 μg), amoxicillin–clavulanic acid (20/10 μg), sulfamethoxazole/trimethoprim (25 μg). S. aureus ATCC 25923 was used as the reference strain for quality control purpose.

3. Results

3.1. Staphylococcal isolates

According to the biochemical tests and MPCR, out of 117 staphylococcal isolates from patients, 96 (82%) were S. aureus. Of 50 personnel, 72 staphylococcal isolates were collected, 20 (28%) of them were S. aureus and rest of them (72%) were coagulase negative staphylococcus. All S. aureus isolates were identified from the different personnel. Rate of the personnel carriage in terms of the S. aureus and MRSA were 40% and 34%, respectively. More than 80% of S. aureus isolates were MRSA while rate of mupirocin resistant S. aureus isolates in patients and personnel were 6% and 70%, respectively.

3.2. MPCR results

The results of the MPCR are shown in Table 1. The rate of the MRSA strains isolated from the patients and personnel was 67% and 34%, respectively. The rate of the mupirocin resistance among patient’s isolates was low (5%). Simultaneous resistance to methicillin and mupirocin was found among 14 (28%) of isolates cultured from personnel. The majority (70%) of MRSA strains that collected from personnel were resistant to mupirocin. The results of the amplification of the femB, mecA and ileS-2 genes are shown in Fig. 1.

3.3. Antibiotic susceptibility

The results of antimicrobial susceptibility patterns of isolates from patients are presented in Table 2. All isolates were susceptible to the linezolid, teicoplanin, and vancomycin. MSSA isolates were more susceptible to the antibiotics and all of them were susceptible to the mupirocin. All MRSA strains that were positive in terms of the mecA gene were also methicillin resistance phenotypically.

4. Discussion

Antibiotic pressure and overuse of broad-spectrum β-lactam antibiotics are the potential factors for the high prevalence of the MRSA in the entire world [4,8]. As compare to the recent studies [4,20], these data showed that the rate of the MRSA is increasing dramatically. The rate of MRSA in this study was near to the studies which were reported from India (78%) and...
Iran (87%) [2,20,21] and higher than the previous studies of Iran (58.5%), Oman (52%) and Turkey (35–43%) [4,22,23]. To the best of our knowledge, Burn Center of Ahvaz Taleghani Hospital [2] is one the healthcare centers that have highest rate of MRSA in Iran. The carriage rate of the MRSA in the personnel (34%) was higher than the previous studies which reported from different regions of Iran [24,25]. Infection control policies and other factors in different healthcare and burn centers may affect the rates of MRSA [4]. Most potential reasons for high rate of MRSA in the hospital presumably are non-use of mupirocin as decolonization or treatment agent, the high number of admitted patients and poor hygiene and infection control. Unlike previous studies [4], our MRSA isolates showed higher rates of resistance to various antibiotics, but they remained susceptible to linezolid, teicoplanin, and vancomycin, similar to other studies [4,26,27].

The high risk of emergence of the mupirocin resistance in MRSA strains has been debated before [28–30]. Importantly, the majority (70%) of personnel’s mupirocin resistant strains in this study were also resistant to methicillin suggesting the possible co-relation between resistances to both antimicrobials among strains of S. aureus. The rate of high-level resistance to the mupirocin was close to the India (5%), Korea (5%), China (6.6%), but higher than the previous studies which reported from Jordan (2.6%), Greek (1.6%), India (2%) and Pakistan (0%) [31–36], and lower than the previous rate reported from Tehran, Iran (25%) [4], respectively.

Many factors affect on the mupirocin resistance but the main reasons are not completely clear. The study showed that previous exposure to mupirocin and previous infection by *Pseudomonas aeruginosa* can be related to the low- and high-level mupirocin resistant *S. aureus* isolates [1]. The co-relation between mupirocin consumption and the rate of resistance to this antibiotic is unclear [12,37]. The origin of the isolate and clinical samples is another factor that could affect the resistance to mupirocin among the population of MRSA strains [4]. *P. aeruginosa* had been reported as the main causative of infection among the patients in Burn Center of Ahvaz Taleghani Hospital during 2003–2004 (9). This may be one of the factors that affected to mupirocin resistance rates in our isolates. Since there was not history of previous usage of this antibiotic in this hospital, these data emphasize on the existence of the mupirocin resistance without intense use of the mupirocin [17].

### Table 1 – Frequency of mecA and iltE-2 genes in the staphylococcal isolates.

<table>
<thead>
<tr>
<th>Gene</th>
<th>P (%) of S. aureus isolates from patients (n = 96)</th>
<th>P (%) of S. aureus isolates from personnel (n = 20)</th>
<th>P (%) of All (n = 116)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mecA</td>
<td>78 (81)</td>
<td>17 (85)</td>
<td>95 (82)</td>
</tr>
<tr>
<td>iltE-2</td>
<td>6 (6)</td>
<td>14 (70)</td>
<td>20 (17)</td>
</tr>
<tr>
<td>mecA + iltE-2</td>
<td>6 (6)</td>
<td>14 (70)</td>
<td>20 (17)</td>
</tr>
</tbody>
</table>

P, positive; N, negative.

### Table 2 – The antimicrobial susceptibility patterns of *Staphylococcus aureus* strains isolated from patients.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>N (%) of MRSA (n = 78)</th>
<th>N (%) of MSSA (n = 18)</th>
<th>N (%) of all (n = 96)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>S: 78 (100) R: 18 (100)</td>
<td>S: 18 (100) R: –</td>
<td>S: 96 (100)</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>S: 78 (100) R: –</td>
<td>S: 18 (100) R: –</td>
<td>S: 96 (100)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>S: 78 (100) R: –</td>
<td>S: 18 (100) R: –</td>
<td>S: 96 (100)</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>S: 78 (100) R: –</td>
<td>S: 18 (100) R: –</td>
<td>S: 96 (100)</td>
</tr>
<tr>
<td>Linezolid</td>
<td>S: 78 (100) R: –</td>
<td>S: 18 (100) R: –</td>
<td>S: 96 (100)</td>
</tr>
<tr>
<td>Mupirocin</td>
<td>S: 67 (86) R: 11 (14)</td>
<td>S: 18 (100) R: –</td>
<td>S: 85 (89)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>S: 14 (18) R: 64 (82)</td>
<td>S: 11 (61) R: 7 (39)</td>
<td>S: 25 (26)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>S: 11 (14) R: 67 (86)</td>
<td>S: 12 (67) R: 6 (23)</td>
<td>S: 23 (24)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>S: 49 (63) R: 29 (37)</td>
<td>S: 13 (72) R: 5 (28)</td>
<td>S: 62 (65)</td>
</tr>
<tr>
<td>Amoxicillin–clavulanic acid</td>
<td>S: 11 (14) R: 67 (86)</td>
<td>S: 8 (44) R: 10 (56)</td>
<td>S: 19 (20)</td>
</tr>
<tr>
<td>Sulfamethoxazole/trimethoprim</td>
<td>S: 26 (33) R: 52 (67)</td>
<td>S: 14 (78) R: 4 (22)</td>
<td>S: 40 (42)</td>
</tr>
</tbody>
</table>

MSSA, methicillin susceptible S. aureus; MRSA, methicillin resistant S. aureus; S, sensitive; R, resistant.

![Fig. 1 – Gel electrophoresis of some of the staphylococcal isolates. M: 100-bp DNA size marker; lane 6: positive control; lanes 2, 4, 5, 7–9: MRSA strains with high-level mupirocin resistance; lanes 1, 3, 10, 11: methicillin resistant coagulase negative staphylococcal isolates with high-level mupirocin resistance.](image-url)
Rate of the carriage of the S. aureus, MRSA and MRSA with high-level mupirocin resistance in the personnel were 40%, 34% and 28%. These data suggests that the personnel are main reservoir for spreading of the dangerous strains of S. aureus which is disaster for the burn patients with immunodeficiency.

In conclusions, the high prevalence of MRSA strains from the patients showed the potential outbreak of the MRSA in the burn center and highlighted the need of antibiotic susceptibility monitoring of MRSA. Moreover being personnel as a main reservoir in terms of MRSA strains with high-level mupirocin resistance emphasizes the screening of the personnel in terms of the MRSA in the healthcare system especially in the burn center.

Conflict of interest

None declared.

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