



Short Communication



## Evaluation of High-Level of Mupirocin Resistance among Clinical Isolates of Methicillin-Resistant *Staphylococcus aureus* from Shiraz, Iran (2008-2009)

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### ABSTRACT

**Background:** The spread of methicillin-resistant *Staphylococcus aureus* (MRSA) is a major concern in medical centers. These isolates are considered in serious infections and nosocomial outbreaks worldwide. Mupirocin is one of the most important antibiotics used topically for the treatment of various staphylococcal and streptococcal skin infections. We aimed to use a combination of phenotypic and genotypic methods to evaluate the prevalence of mupirocin resistance among MRSA clinical isolates which had previously been collected during the period 2008-2009 in Shiraz, Iran. **Methods:** This study was performed on a total of 167 clinical isolates of MRSA from Shiraz teaching hospitals. The isolates were identified as *S. aureus* using standard microbiologic procedures and confirmed as MRSA isolates by 30 µg cefoxitin discs and *mecA* gene detection. All isolates were investigated for the mupirocin resistance by mupirocin discs 5 µg and the presence of *mupA* gene by PCR. **Results:** Antibacterial susceptibility tests against mupirocin disc 5 µg and PCR analysis for totally 167 MRSA clinical isolates showed no resistance to mupirocin. **Conclusion:** In summary, fortunately regarding to absence of resistance to mupirocin among all the studied MRSA isolates, this resistance seems is not a threatening factor in studied hospitals. However, generalized our findings to whole hospitals wards and Shiraz general population afford larger sample size and periodic surveillance in further studies for detecting mupirocin resistance.

### Introduction

*Staphylococcus aureus* is Gram-positive bacterium found in many parts of human hosts.<sup>1</sup> *S. aureus* is one of the most important isolated pathogens in both community and clinical setting.<sup>1</sup> *S. aureus* as an opportunistic pathogen is one of the most common causative agent of nosocomial infections such as sepsis, pneumonia and postoperative surgical wound infections which can lead to multiplicity of self-limiting and even life-threatening diseases.<sup>1,2</sup>

Nowadays, in spite of the great advanced in antibacterial agents and health care, the spread of methicillin-resistant *S. aureus* (MRSA) is major concern in medical centers.<sup>1</sup> These isolates are considered in serious infections and nosocomial outbreaks worldwide.<sup>1,3</sup> Early detection and immediate treatment of MRSA infections is necessary during its potentially devastating effects.<sup>1</sup>

The expression of PBP2a encoded by the *mecA* gene

that located on a mobile genetic element called the staphylococcal cassette chromosome lead to methicillin resistance in *S. aureus*.<sup>4</sup>

Mupirocin (pseudomonic acid A) with a unique chemical structure is one of the most important antibiotics often used topically for the treatment of various staphylococcal and streptococcal skin infections.<sup>5</sup> Mupirocin ointment has shown encouraging results in skin infections and have advantages compared to other similar antibiotics including little systemic absorption, rapidly inactivation and excretion via urine and having no cross-resistance risk to other antibiotics.<sup>6</sup> Mupirocin has successfully been used to eliminate nasal carriage of *S. aureus*, particularly MRSA isolates.<sup>2</sup> Moreover, nosocomial infection rates have been shown to be significantly reduced by regular topical application of mupirocin.<sup>7,8</sup>

The mechanism of the action of mupirocin is inhibition

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of protein synthesis by inactivating of isoleucyl-tRNA synthetase (IRS).<sup>9,10</sup> However, extensive use in long term has led to the emergence of mupirocin resistance in MRSA, methicillin- susceptible *S. aureus* (MSSA) and glycopeptide -intermediate *S. aureus*.<sup>2,9</sup>

There are two types of mupirocin resistance: low level (MuL), with minimum inhibitory concentrations (MICs)  $\geq 8$   $\mu\text{g}/\text{mL}$  to  $\leq 256$   $\mu\text{g}/\text{mL}$ , and high level (MuH) with MIC  $\geq 512$   $\mu\text{g}/\text{mL}$ .<sup>11</sup> MuL is due to mutations in the chromosomal IRS, whose clinical significance is unclear. MuH results from the uptake of an additional IRS that confers resistance to mupirocin. Gene encoding of this type of resistance (*ileS-2*, or *mupA*) is located on plasmids, but is occasionally present on the chromosome.<sup>11</sup> These strains with MuH and sometimes MuL phenotype are not eradicated by topical prescription of mupirocin and have been associated with treatment failure with mupirocin.<sup>2,9</sup>

Mupirocin resistant MRSA in some groups are more critical such as burn patients who are vulnerable to superficial infections. Previously, high-level of mupirocin resistance was reported among burn patients in a burn center from Ahvaz city, Iran.<sup>10</sup> In another survey from west of Iran (Kermanshah city), mupirocin resistant *S. aureus* strains among hospitalized nasal carriers were not detected and the range of mupirocin MICs was determined at low level.<sup>12</sup>

Previous reports indicated to ability of MRSA strains in colonization in many sites of human body and subsequently associated with wide range of infections.<sup>1,9</sup> This issue and potential risk of mupirocin resistant strains emphasizes the screening of mupirocin resistance not even in specific groups or body site, but in other clinical samples as well.

To the best of our knowledge in our area Shiraz city, south-west of Iran, no specific study has been done on mupirocin resistance. Therefore, we aimed to use a combination of phenotypic and genotypic methods to evaluate the prevalence of mupirocin resistance among MRSA clinical isolates which had previously been collected during the period 2008-2009 in Shiraz, Iran.

## Materials and Methods

### Study design and bacterial isolates

The present study conducted on 167 nonduplicate MRSA clinical isolates which were obtained from Shiraz teaching hospitals (Nemazee, Faghihi, Beheshti, Ghotbedin and Chamran) within one year from October 2008 to 2009. These bacteria were isolated from different clinical specimens such as blood, pus, wound, urine, etc. To determine MRSA isolates, 30  $\mu\text{g}$  cefoxitin discs (Mast Group, Ltd, U.K) were used and then confirmed by molecular methods. After recovery of frozen bacteria from  $-70$  °C and growth on the tryptic soy agar (Merck, Germany), the isolates were again identified as *S. aureus* based on colonial morphology, Gram staining, coagulase and DNase tests. For phenotypic determination of mupirocin-resistant isolates, mupirocin discs 5  $\mu\text{g}$  (Mast Group,

Ltd, U.K) were used by disc diffusion on Mueller-Hinton agar (Merck, Germany). After 24 h incubation at 37 °C, the results were interpreted as described by Finlay *et al.*<sup>13</sup> *S. aureus* ATCC 25923 as a methicillin-sensitive *Staphylococcus aureus* and mupirocin sensitive was used as the control strain in antibacterial susceptibility testing.

### Molecular assess

In molecular method, we used DNA genomic samples of 167 MRSA isolates. PCR were performed with previously described primers for detection of *mecA* and *mupA* among isolate.<sup>14,15</sup> PCR assay for detection of desired genes was optimized separately in a volume of 25  $\mu\text{L}$  using the described primers following standard time and thermal program (Initial denaturation 95 °C 5 min, followed by 30 cycles of denaturation at 95C for 30 sec, annealing 50 °C for 45 sec, extension 72 °C for 1 min and a final extension at 72 °C for of 5 min) with the DNA Thermal Cycler 5530 (Ependrof master, Germany). The amplicons were assessed by 1% agarose gel electrophoresis and visualized on an UV transilluminator. In each gel the positive and negative controls were included. One clinical *S. aureus* isolate which had become positive for presence of *mupA* was used as positive control in the current study. DNA sequencing was performed by BIONEER Co, South Korea.

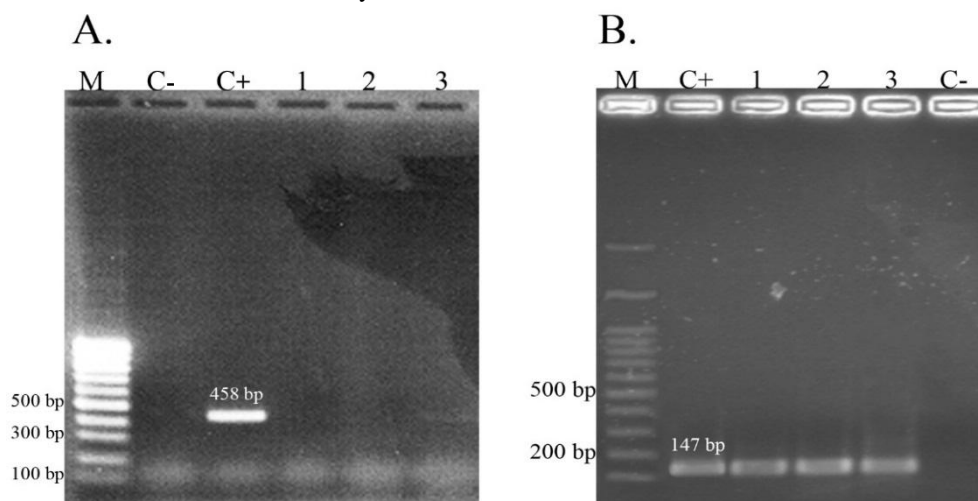
## Results

Of totally 400 *S. aureus* which isolated within one year from different clinical samples, 263 (65.8%) isolates were obtained from men and 137 (34.2%) from women. Overall, frequency of MRSA isolates were 41.8% (167/400). The majority of MRSA isolates obtained from burn infections (68%) and followed by pneumonia (56.6%) and wound infections (51.4%). Results of antibacterial susceptibility tests for MRSA isolates showed no resistance against mupirocin disc 5  $\mu\text{g}$ . As well, from totally 167 MRSA clinical isolates which were analyzed by PCR no desired amplification were seen. In negative control DNA sample, no amplification product was produced, but a single sharp of approximately 458 bp amplicon was shown in the positive control sample (Figure 1).

## Discussion

MRSA strains are the most frequent pathogens causing nosocomial infections throughout the world and their increasing resistance to  $\beta$ -lactams has become an important medical concern.<sup>9,11</sup> It has been shown that mupirocin is the best drug for decolonization of staphylococcal and streptococcal infections.<sup>2,13</sup> Although mupirocin resistance in various regions of the world is low,<sup>16</sup> there are documents indicating that this resistance is increasing.<sup>2</sup> In the current study, we carried out a combination of phenotypic and molecular tests to detect mupirocin resistance among Iranian MRSA clinical isolates. Hopefully, none of the tested

MRSA isolates produced an inhibition zone of  $\leq 14$  mm in diameter and an amplicon of the expected size. In fact the results obtained by the disc diffusion were in agreement with those of the PCR assay. It is



**Figure 1. A.** Representative gel image of *mupA* gene detection by PCR. M: 100 bp ladder; C-: negative control (*S. aureus* ATCC 25923); C+: positive control for *mupA* gene (458 bp); lane 1-3: clinical isolates of MRSA. **B.** Representative gel image of *mecA* gene detection by PCR. M: 100 bp ladder; C-: negative control (*S. aureus* ATCC 25923); C+: positive control for *mecA* gene (147 bp); lane 1-3: clinical isolates of MRSA.

In the present survey, susceptibility of our isolates was confirmed by no amplification of *mupA*. Moreover, according to the study conducted by Fuchs *et al.*,<sup>18</sup> different low content discs (5, 10 or 20 $\mu$ g) are suitable and reliable for detecting mupirocin resistant staphylococci; however, these discs cannot discriminate between MuL and MuH strains. In our study, all the isolates were sensitive against mupirocin 5  $\mu$ g discs and did not need to be checked out with 200  $\mu$ g discs for determining of MuH isolates. This observation was demonstrated by Palepou *et al.*<sup>17</sup>

Abbasi-Montazeri *et al.* in a hospital survey from south-west of Iran (Ahvaz city) indicated to notable rate of 34% mupirocin resistance among their recovered MRSA isolates from burn patients.<sup>10</sup> In the study of Shahsavan *et al.* from north of Iran (Tehran city), the rate of mupirocin resistant MRSA strains obtained from burnt patients was 68%.<sup>19</sup> Additionally, in the mentioned study there was no concurrence between phenotypic and genotypic technique results. Compared to such high rate of mupirocin resistant MRSA strains from burn patients, Mohajeri *et al.* same as our study showed no mupirocin resistance in *S. aureus* isolates obtained from hospitalized nasal carriers from west of Iran (Kermanshah city).<sup>12</sup> Moreover, in the only study conducted in Shiraz,<sup>20</sup> all the *S. aureus* clinical isolates including 156 MRSA, were susceptible to mupirocin which is compatible with our observation. It seems that lack this resistance in this region of Iran is due to the low or lack of using this drug in our clinics. On the other hand, perhaps this discrepancy is related to the type of clinical sample. The origin of isolates and clinical specimens is

established that the only method differentiating between MuL and MuH among *S. aureus* clinical isolates is MIC determination and PCR for *mupA*.<sup>17</sup>

mentioned as the factors that affect mupirocin resistance among MRSA strains.<sup>10</sup> In Iran, the high frequency of mupirocin resistant MRSA was mostly associated with burn wards,<sup>19</sup> while in our study the MRSA isolates were obtained from different clinical samples other than burn wards. Finally, beside the limited sample size which could not generalize to whole Shiraz population, lack of MIC investigation was another limitation of present study.

### Conclusion

In summary, regarding to absence of resistance to mupirocin among all the studied MRSA isolates, fortunately this resistance seems is not a threatening factor in studied hospitals. However, generalized our findings to whole hospitals wards and Shiraz general population afford larger sample size and periodic surveillance in further studies for detecting mupirocin resistance. This is one of the initial studies of mupirocin resistance among *S. aureus* isolates in Shiraz, where probably the usage of this drug is very rare or limited. Therefore, this negative result may indicate this issue; however, this matter doesn't rule out the necessity of doing periodic monitoring.

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**Conflict of interests**

The author claims that there is no conflict of interest.

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