



Frequency of Mupirocin Resistant *Staphylococcus aureus* Strains Isolated From Nasal Carriers in Hospital Patients in Kermanshah

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ABSTRACT

Background: *Staphylococcus aureus* is a major nosocomial pathogen world wide. Mupirocin plays a crucial role in strategies designed to control outbreaks of *S. aureus*.

Objectives: The aim of this study was to determine the frequency of mupirocin resistance in *S. aureus* strains isolated from nasal carriers among the hospitalized patients at Kermanshah Hospital, Iran.

Patients and Methods: A total of 174 *S. aureus* isolates (sensitive and resistant to methicillin) were collected from the nasal anterior nares of hospitalized patients. All isolates were tested for mupirocin susceptibility by a disc diffusion method. The minimum inhibitory concentration (MIC) was determined by an E-test and they were also analyzed by a PCR for the presence of *ileS-1* and *ileS-2* genes.

Results: Utilizing the disc diffusion agar method, E-test and PCR, all of the *S. aureus* strains tested were susceptible to mupirocin. In this study, the range of mupirocin MICs was determined to be between 0.064 and 4 µg/ml. There was a significant association between MIC observed and multi-drug resistant (MDR) carriage (*P* value 0.04), and resistance to oxacillin (*P* value 0.004).

Conclusions: This is a report of an initial survey of mupirocin resistance in *S. aureus*, in Kermanshah where the use of mupirocin is still limited. Perhaps the sensitivity of all isolates to mupirocin in this study is due to the less common usage of this antibiotic, especially in the form of nasal and site sample collections.

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► Implication for health policy/practice/research/medical education:

Mupirocin resistance has been associated with failure to clear MRSA from nasal carriage patients.

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1. Background

Staphylococcus aureus has been recognized as the main etiological agent and the most frequent microorganism in community-acquired and hospital-acquired infections (1). Nasal colonization with *S. aureus* is common and it is an important step in the pathogenesis and spread of *S. aureus* infections, these strains provide a reservoir for infection in other sites such as; surgical-site and blood-stream infections. In certain subgroups, such as; frequently hospitalized people, senile and immune compromised patients, colonization with *S. aureus* occurs more frequently (2). Currently, the health problems associated with this microorganism have become more serious due to an increasing incidence of methicillin-resistant *S. aureus* (MRSA) (3). As an antibiotic, mupirocin (pseudomonic acid A) is an analogue of isoleucine that inhibits protein synthesis by competitively binding to the enzyme isoleucyl-tRNA synthetase. It was first introduced in the UK as one of the most effective topical antibiotic that is active against gram-positive pathogens, as well as some gram-negative bacteria and it is used for the eradication of *S. aureus* in the nasal carriage (4, 5).

Of the two mupirocin-resistant phenotypes, the low-level resistant strain (MIC 8-256 µg/ml) is more common with a point mutation by the isoleucyl-tRNA synthetase gene (*ileS-1*) for the target enzyme, and a high-level of mupirocin resistance (MIC ≥ 512 µg/ml), from the acquisition of a plasmid carrying a new gene, *ileS-2* or (*mupA*), it encodes an alternate isoleucyl-tRNA synthetase (6, 7). Nasal carriage therapy with mupirocin ointment appears to be effective in reducing the onset and severity of infections at surgical sites (1).

2. Objectives

At present, the prevalence of these resistant organisms from the nasal carriage of patients in the Kermanshah Hospital, which is the largest hospital in western Iran, is still unknown.

3. Patients and Methods

3.1. Specimen Collection and Bacterial Detection

Nasal swabs were taken five times by rotating a sterile cotton swab, moistened with sterile saline, in the vestibule of both anterior nares of hospitalized patients in different wards (ICU, CCU, surgery, internal medicine, gynecology, infection, heart, pediatric, infants and hemodialysis) of the Kermanshah Hospital which is the largest hospital in western Iran, between October 2009 and August 2010 (6, 7). Trypticase soy broth (TSB) was used as a transport medium. Swabs were transported in Amies medium and processed within two hours of collection. The swabs were put directly onto mannitol salt agar (Merck, Germany) and sent to the laboratory and incubated at 35° C in a humidified incubator for 48 h. Strains that

produced yellow colonies on the MSA plate, were subcultured on a blood agar plate (Merck, Germany), for further characterization.

The staphylococcal isolates were identified by *conventional* methods, e.g. colonial morphology, gram staining characteristics, production of catalase, coagulase tube method using rabbit plasma, DNase in tube tests and other biochemical tests (8). A total of 174 non-repetitive *S. aureus* isolates (sensitive and resistant to methicillin) were collected.

3.2. Antibiotic Susceptibility Testing

Screening for methicillin resistance was determined by a Kirby-Bauer disk diffusion test, according to the guidelines published by the Clinical and Laboratory Standards Institute. Using a 30 µg cefoxitin disk (MAST, UK), the disk was placed on Mueller-Hinton agar (Merck, Germany) and incubated for 24 h at 35° C following CLSI guidelines (9).

In determining resistance to mupirocin as the first step, all isolates were screened by the disk diffusion method using a 5µg mupirocin paper disk (MAST). An isolate with inhibition zones ≥ 14 mm around the disk were designated as sensitive, but these discs failed to distinguish between low level and high level resistance (9, 10). To analyze the sensitivity patterns of the mupirocin strains more precisely, MICs for *S. aureus* isolates to mupirocin were assessed using E-test® mupirocin strips (AB-BIO-DISK, Solna, Sweden) according to the manufacturer's instructions. The E-test strip was applied onto each plate of Mueller-Hinton agar that was inoculated with a suspension of isolates to the optical density of a 0.5 McFarland standard with sterile forceps. Following incubation at 35° C for 24 h. E-test MIC values were read by the operator at the point where the bottom of the inhibition zone intersected with the E-test strip. Strains were considered to be susceptible if the MIC value was ≤ 4 mg/L and levels of mupirocin resistance were defined as low-level with MIC 8-256 mg/l and high-level with MIC ≥ 512 mg/L (7). For both MSSA and MRSA, we defined multi-drug resistant (MDR) isolates as those that were resistant to 3 different antibiotics, i.e. co-trimoxazole, ciprofloxacin and erythromycin (11).

3.3. Polymerase Chain Reaction (PCR)

All of the strains were analyzed for the presence of the *ileS-1* and the *ileS-2* (*mupA*) gene by boiling the bacterial cells and PCR (12, 13). To extract the DNA, the isolated staphylococci were cultured on blood agar (Merck, Germany) for 24 hours. Subsequently, a loop full of bacterial cells from each sample were removed and resuspended in 250 µl of sterile distilled water and the suspension incubated in a 90° C heat block for 15 min. Centrifugation followed (7 500 × g, 5 min) and the supernatant containing the staphylococcal DNA extract was transferred into

new test tubes and frozen for later PCR amplification. Five microlitres of the extracted DNA was transferred to 20 μ l of the PCR amplification mix consisting of; 2.5 μ l of 10 X buffer, 1.5 mM of $MgCl_2$, 1.5 U of *Taq* polymerase, 1.25 μ l of dNTPs and 1.5 μ l of each primer. To identify point mutations of the *ileS-1* gene, the 690-bp product was amplified using a primer pair *Imr1* (5'-GTA AAT CTT TAG GTA ATG TGA TTG TAC-3') and *Imr2* (5'-TCT TCT TTA ACA TGT GGT GTA TGA GA-3') (13). To detect the *ileS-2* gene (*mupA* gene), a 456-bp region in the *ileS-2* gene was amplified by PCR, using a primer pair *Mup1* (5'-TAT ATT ATG CGA TGG AAG GTT GG-3') and *Mup2* (5'-AAT AAA ATC AGC TGG AAA GTG TTG-3') (14). Cycling parameters were 94° C for 5 min followed by 30 cycles, denaturation at 94° C for 30 s, annealing at 52° C for 30 s and extension at 72° C for 30 s, and a final 5 min incubation at 72° C. Following PCR amplification, the amplification (PCR) products were analyzed by agarose gel electrophoresis (1 \times Tris-Boric acid-EDTA, 1.2% agarose, 75V, 70 min) stained with ethidium bromide and the amplicons were visualized using a UV light box.

S. aureus ATCC (25923) was used as a reference strain for a Mup quality control during susceptibility testing and DNA *S. aureus* jji1 from Marcia Giambiagi-deMarval (Brazil) were mupirocin resistant and used as the reference strain quality control for PCR (12).

3.4. Statistical Analysis

The data was entered in Microsoft Access XP software and exported into SPSS statistical software, version 16.0, which was used for the data analyses. The categorical data were compared using a chi-squared test or Levene's test. All *P* values were two-sided with *P* < 0.05 being considered significant.

4. Results

Of the 174 patients studied, 93 (53.4%) were male and 81 (46.6%) were female, ranging in age from one to 84 and from one to 83 years, respectively. Mean age was 39.47 \pm 25.92 and 45.26 \pm 24.37 for males and females, respectively. The MIC of the individuals was not associated significantly with age. (*P* value 0.859) With the disc diffusion agar method, all of the 174 *S. aureus* strains tested were susceptible to mupirocin. Single PCR of each gene was conducted for all of the isolated, but none of the strains contained *ileS-1* and *ileS-2*.

In this study, the E-test range of mupirocin MICs was determined to be between 0.064 and 4 μ g/mL. A total of 108 (62.2%) strains had MICs less than 1 μ g/mL and 66 (37.8%) strains had MICs more than 1 μ g/mL. Of the 174 isolates, 92 (52.8%) were found to be methicillin resistant and 82 (47.2%) were methicillin susceptible. Their mean MIC was 1.33 \pm 1.38 and 0.81 \pm 0.95 for the MRSA and MSSA strains respectively. In the Levene's test, the MIC of the individuals was significantly associated with resistance to oxacillin (*P* value 0.004). Of the 174 isolates, 99 (56.9%) were multi-

drug resistant. In the Levene's test, their mean MIC was 1.24 \pm 1.31 and 0.89 \pm 1.06 respectively for the MDR positive and MDR negative isolates (*P* value 0.04).

5. Discussion

Based on our findings, none of the *S. aureus* isolates (MRSA and MSSA) were mupirocin resistant and none of the demographic characteristics of the carriers or antibiotic resistance patterns or even the source of the isolates (hospital/ community acquired) showed any determinant role in mupirocin sensitivity. The MIC of 16 (9.2%) of the strains was 4 μ g/mL which is very close to a low level resistance (8 μ g/mL). The emergence of mupirocin resistance among *S. aureus* isolates has been clearly defined in many parts of the world at different frequencies: Spain 11.3%, USA 13.2%, Trinidad Tobago 26.1%, China 6.6%, India 6%, Turkey 45% and Korea 5%, however, it does appear to be increasing worldwide (2, 4, 5, 10, 14-16).

In the two reports from Iran, the prevalence of such resistant strains was 2.7% and 0% (1, 7). It can be assumed that the absence of resistant strains in our study may be related to two factors: the rare use of mupirocin as an empiric therapy in Kermanshah and lack of clinical samples. In Iran, although mupirocin is not applied to eliminate *S. aureus* nasal colonization in patients as part of infection control measures, it is infrequently used for the treatment of skin infections. Rapid resistance to mupirocin occurs among strains of *S. aureus* isolated from hospitals. Therefore, monitoring for mupirocin resistance in *S. aureus*, especially in MRSA, is necessary to evaluate the usefulness of mupirocin in both the treatment of staphylococcal infections and infection control.

The observed MIC of 4 in this study, therefore, recommends a continuous surveillance plan in our area. To date, mupirocin has not yet been used for the eradication of *S. aureus* in nasal carriers in our hospital. But a local application of mupirocin ointment has been shown to eliminate MRSA nasal colonization in both patients and hospital staff. The full susceptibility of *S. aureus* to mupirocin observed in this study indicates that it is effective for the treatment of *S. aureus* infections in our hospital. It seems that antibiotic use led to the emergence of resistant strains (10, 16). However, there are other studies which have reported the isolation of mupirocin-resistant *S. aureus* from patients who had not taken mupirocin (5, 7, 15).

Mupirocin-resistant strains were often resistant to other antimicrobial agents. For example there is a relationship between methicillin resistance and resistance to mupirocin, but in this study such a relationship has not been proven (14). However, MIC appears to be significantly greater among MRSA than in MSSA strains (*P* value 0.004). Our study showed that 80% of the strains with MIC 4 μ g/mL were multidrug-resistant (*P* value 0.04). All of the studied strains showed the same results in the disc

diffusion agar method, E-test and PCR. So, we think that there is no significant difference between these methods in the detection of mupirocin resistance.

In conclusion, the present study revealed that our studied *S. aureus* strains were completely sensitive to mupirocin and the prevalence of mupirocin resistant *S. aureus* strains in the nasal carrier patients was similar to the prevalence that was reported by the other study in our country (1, 7). Further studies in different regions of Iran will show the level of this similarity for consideration in national infection control programs. Nonetheless, the need for continuous surveillance remains important. Detecting the introduction of mupirocin-resistant strains, especially in hospital fields which have shown higher levels of resistance to mupirocin and multiple reports of higher rates of resistance to mupirocin in methicillin resistance isolates, is the rationale for such surveillance.

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