ORIGINAL RESEARCH

Phenotypic and genotypic determinants of mupirocin resistance among *Staphylococcus aureus* isolates recovered from clinical samples of children: an Iranian hospital-based study

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Backgrounds: The aim of this study was to evaluate both phenotypic and genotypic determinants of mupirocin resistance among methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin susceptible *S. aureus* (MSSA) strains recovered from different clinical samples of children who were admitted to the Children's Medical Center (CMC) Hospital, Tehran, Iran.
Materials and methods: A total of 120 clinical isolates of *S. aureus* were collected from the microbiology laboratory of CMC Hospital. Antimicrobial susceptibility of the isolates to different antimicrobial agents was determined by disk diffusion method. The methicillin resistance phenotype (MRSA) was identified using a 30 μg cefoxitin disk. The minimum inhibitory concentration (MIC) of mupirocin was determined by broth microdilution method. Strains with mupirocin MIC between 8 and 256 μg/mL were considered as low-level mupirocin resistant (LLMR), and strains with an MIC≥512 μg/mL were considered as high-level mupirocin resistant (HLMR). The presence of genes encoding HLMR (ie, *mup*A and *mup*B genes) was evaluated by PCR method.
Results: Four out of 120 isolates (3%) had mupirocin MIC≥512 μg/mL and were HLMR; however, no LLMR isolate was detected. Fifty-two isolates (43%) were MRSA, and there were no differences in the distribution of mupirocin resistance among MRSA and MSSA isolates (*P*>0.05). The PCR method identified *mup*A gene in two out of four HLMR isolates, and *mup*B gene was not detected in any HLMR isolates.
Conclusion: Because of discrepancies between the phenotypic and genotypic patterns of mupirocin resistance and due to the avoidance of false-negative results, it is better to determine the mupirocin resistance by both antibiotic susceptibility tests and PCR method. Considering the increasing need of mupirocin for the control of *S. aureus* infections, continuous checking of its susceptibility status is necessary.
Keywords: methicillin-resistant *Staphylococcus aureus*, mupirocin, PCR, children

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant pathogen contributing in healthcare- and community-associated infections all over the world.[1] These isolates are resistant to a wide variety of currently accessible antibacterial agents such as macrolides, lincosamides, aminoglycoside, and all beta-lactams. The occurrence of multidrug resistance (MDR) among MRSA strains is a serious dilemma in treatment and control of their infections.[2]

Mupirocin (pseudomonic acid A) is a topical ointment that is broadly used for the treatment of staphylococcal skin infections and is effective for nasal decolonization

of MRSA.[3–5] The mechanism of action of mupirocin involves attaching to the bacterial isoleucyl–tRNA synthetase and interfering with protein synthesis.[6] Widespread and long-term use of this medicine led to the emergence of mupirocin-resistant organisms.[7]

According to the results of minimum inhibitory concentration (MIC) test, two kinds of mupirocin resistance have been explained. Low-level mupirocin resistance (LLMR) with an MIC between 8 and 256 µg/mL is mediated by point mutations in tRNA synthetase chromosomal gene (*ile*S-1).[4,6–9] This mutation is stable and nontransferable[10] and its related resistance (ie, LLMR) is not clinically relevant because mupirocin is a topical antibiotic and has high concentrations on the infection site.[11] High-level mupirocin resistance (HLMR) with an MIC ≥512 µg/mL is the result of the acquisition of plasmid-borne resistance genes *mup*A (also known as *ile*S-2) or *mup*B. Both the genes encode additional isoleucyl-tRNA-synthetases that are not sensitive to repression by mupirocin.[6,10,12,13] The *mup*A encoding plasmid may also carry resistance genes to other antibiotics. So it is likely that the application of mupirocin not only results in mupirocin resistance but also leads to the increasing resistance to the other antibiotics.[6]

The aim of this study was to evaluate both the phenotypic and genotypic determinants of mupirocin resistance among MRSA and MSSA strains recovered from different clinical samples of children who were admitted to the Children's Medical Center (CMC) Hospital, Tehran, Iran.

Materials and methods

CMC Hospital is one of the most experienced subspecialized hospitals in Iran and offers high-quality and specialized therapeutic services to neonates, infants, and children throughout the country and region. Our center consists of about 20 specialty and subspecialty wards, including emergency medical services, electronic intensive care unit, infectious diseases, hematology, nephrology, hemodialysis, endocrinology, gastrointestinal disease, endoscopy, neonatology, neonatal intensive care unit, pediatric intensive care unit, immunology, rheumatology, neurology, psychiatry, cardiology, open heart intensive care unit, coronary intensive care unit, respiratory disease, surgery, orthopedic, cardiac, ENT, neurosurgery, urology, and operation theaters. This center was selected as the hub of excellence in pediatrics in 2008 by the Iranian Ministry of Health, Treatment and Medical Education which provides subspecialty care for more than 1,500 patients monthly.

Bacterial strains

During a cross-sectional study in 2016, a total of 120 nonduplicate clinical isolates of *S. aureus* were recovered from wound, trachea, eye, blood, inguinal region, abscess, lymph nodes, and bone marrow samples of the children admitted to CMC hospital. All of the isolates were re-identified using conventional confirmatory tests, such as Gram stain, catalase and coagulase production, DNase, and mannitol fermentation.[14]

Antibiotic susceptibility testing

The antibiotic susceptibility of the isolates was evaluated according to the guidelines published in 2016 by Clinical and Laboratory Standards Institute (CLSI).[15,16] The following antibiotic disks were applied in disk diffusion agar test: amikacin (30 µg), azithromycin (15 µg), clindamycin (2 µg), moxifloxacin (5 µg), penicillin (10 U), tetracycline (30 µg), linezolid (30 µg), cefazolin (30 µg), quinupristin–dalfopristin (15 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), chloramphenicol (30 µg), minocycline (30 µg), cloxacillin (1 µg), cefoxitin (30 µg), rifampicin (5 µg), and ciprofloxacin (5 µg). All disks were purchased from Mast Co., UK, and *S. aureus* ATCC 25923 was used for quality control of the test.

The methicillin resistance phenotype (MRSA) was identified using a 30 µg cefoxitin disk, and the results were interpreted based on the CLSI standards. The MICs of vancomycin and mupirocin (Mast Co., UK) were determined by E-test and broth microdilution methods, respectively. Isolates with an MIC≤2 µg/mL were sensitive to vancomycin. Strains with their mupirocin MICs between 8 and 256 µg/mL were considered as LLMR, and isolates with an MIC≥512 µg/mL were considered as HLMR.

Detection of HLMR encoding genes

Genomic DNA of HLMR isolates was extracted using the phenol-chloroform-isoamyl alcohol method as described previously.[17] The *mup*A and *mup*B genes were amplified by PCR method using the following oligonucleotide primers, mupA-F: TATATTATGCGATGGAAGGTTGG, mupA-R: AATAAAATCAGCTGGAAAGTGTTG, mupB-F: CTAGAAGTCGATTTTGGAGTAG, and mupB-R: AGTGTCTAAAATGATAAGACGATC.

The PCR mixture for the amplification of these genes (final volume of 25 µL) consisted of 2.5 µL of reaction buffer, 0.5 µL of 100 mM MgCl$_2$, 2.5 units of *Taq* DNA polymerase, 0.5 µL of 50 µM dNTP, 0.5 µL of 10 pMol primer, 19.8 µL of distilled water, and 0.5 µL of DNA template.

The amplification was performed using the following conditions: initial denaturation step at 94°C for 5 minutes, 30 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, and a final extension at 72°C for 5 minutes.

Statistical analysis

Statistical analysis of the results was performed by the statistical package SPSS 16.0 (SPSS Inc. Chicago, IL, USA). Variables were compared by chi-square test and Student's t-test. The level of significance was set at $P<0.05$.

Ethics approval and consent to participate

The study protocol was reviewed and approved by the Pediatric Infectious Disease Research Center of Tehran University of Medical Sciences.

Results

The demographic and clinical characteristics of the patients are listed in Table 1. Fifty-six percent of the patients were male (n=67) and 44% were female (n=53). The wound was the most frequent site of infection (35%, n=42), whereas the bone marrow was the least frequent site (3%, n=4). Most of the strains (33%, n=40) were isolated from the infectious department of the hospital and the least isolation rate was related to oncology ward (3%, n=4).

The antibiotic susceptibility patterns of the isolates are presented in Table 2. According to the results, penicillin was

Table 1 Demographic and clinical data of children with Staphylococcal infection

Gender	N (%)
Male	67 (56)
Female	53 (44)
Specimen source	
Wound	42 (35)
Blood	24 (20)
Eye	16 (13)
Inguinal region	13 (11)
Trachea	11 (9)
Abscess	5 (4)
Lymph nodes	5 (4)
Bone marrow	4 (3)
Hospital ward	
Neonatal intensive care unit	17 (14)
Rheumatology	8 (7)
Oncology	4 (3)
Surgical	25 (21)
Pediatric intensive care unit	10 (8)
Infectious	40 (33)
Emergency	10 (8)
Gastrointestinal	6 (5)

the least effective antibiotic and had the highest resistance rate (96%, n=115). In contrast, vancomycin (0%), amikacin (1.66%, n=2), quinupristin–dalfopristin (2%, n=2), rifampicin (2%, n=2), linezolid (3%, n=4), and cefazolin (10%, n=12) were the most effective antibiotics. Four out of 120 isolates (3%) were resistant to mupirocin, and their MIC was ≥512 µg/mL (ie, HLMR) and no LLMR isolate was detected (Table 3).

A total of 52 isolates (43%) were resistant to methicillin (MRSA). There were significant statistical differences between the resistance rates of MRSA and methicillin sensitive (MSSA) isolates to amikacin, cefazolin, penicillin, cloxacillin, and ciprofloxacin ($P<0.05$); and MRSA isolates showed higher rates. However, there were no significant statistical differences between the resistance rates of these two groups against other tested antibiotic ($P>0.05$) (Table 3). Vancomycin (0%), linezolid (2%), mupirocin (4%), quinupristin–dalfopristin (6%), and rifampicin (6%) were the most effective antibiotics against MRSA isolates. There were no differences in the distribution of mupirocin resistance among MRSA and MSSA isolates ($P>0.05$).

The PCR method identified *mup*A (*ile*S-2) gene in two out of four HLMR isolates, and *mup*B gene was not detected in any HLMR isolates.

Discussion

S. aureus is an important human pathogen responsible for a wide range of infections such as skin and soft tissue infections, endovascular infections, pneumonia, septic arthritis, endocarditis, osteomyelitis, foreign-body infections, and sepsis.[18,19] Administration of mupirocin is an old preventive method for eradication of *S. aureus* carriage in patients undergoing surgery or dialysis and patients hospitalized in intensive care units.[6] Likewise, the use of mupirocin is a common approach for decolonization of nasal MRSA and control of its spread in hospitals.[10] It is also a good choice for the treatment of mild skin infections caused by MRSA strains.[20] However, the emergence of resistance to this drug limits its efficiency. In this study, the prevalence of both phenotypic and genotypic determinants of mupirocin resistance among MRSA and MSSA strains retrieved from children with a variety of staphylococcal infections was evaluated.

In this study, the isolation rate of *S. aureus* was higher in male patients (male to female ratio: 1.2). This was similar to the rate observed in the previous study conducted in CMC hospital.[21] In contrast to our study that wound was the most common isolation site (35%), in the study conducted by

Table 2 The antibiotic susceptibility patterns of *Staphylococcus aureus* isolates

Antibiotic	Sensitive N (%)	Intermediate N (%)	Resistant N (%)
Amikacin	110 (91.66)	8 (6.66)	2 (1.66)
Azithromycin	52 (43)	3 (3)	65 (54)
Moxifloxacin	80 (77)	2 (2)	22 (21)
Tetracycline	90 (75)	–	30 (25)
Vancomycin	120 (100)	–	–
Cefazolin	108 (90)	–	12 (10)
Trimethoprim-sulfamethoxazole	86 (72)	–	34 (28)
Quinupristin–dalfopristin	114 (95)	4 (3)	2 (2)
Chloramphenicol	84 (70)	29 (24)	7 (6)
Clindamycin	67 (56)	33 (27.5)	20 (16.5)
Penicillin	5 (4)	–	115 (96)
Linezolid	116 (97)	–	4 (3)
Minocycline	103 (86)	4 (3)	13 (11)
Cloxacillin	83 (69)	–	37 (31)
Methicillin	68 (57)	–	52 (43)
Rifampicin	111 (92)	7 (6)	2 (2)
Ciprofloxacin	79 (77)	3 (3)	21 (20)

Table 3 The antibiotic susceptibility patterns of *Staphylococcus aureus* isolates

Antibiotic	N (%) of MSSA (n=68)		N (%) of MRSA (n=52)	
	Sensitive	Resistant	Sensitive	Resistant
Amikacin	68 (100)	0 (0)	42 (81)	10 (19)
Azithromycin	31 (46)	37 (54)	21 (40)	31 (60)
Moxifloxacin	55 (81)	13 (19)	41 (79)	11 (21)
Tetracycline	51 (75)	17 (25)	39 (75)	13 (25)
Vancomycin	68 (100)	0 (0)	52 (100)	0 (0)
Cefazolin	68 (100)	0 (0)	40 (77)	12 (23)
Trimethoprim-sulfamethoxazole	53 (78)	15 (22)	33 (63.5)	19 (36.5)
Quinupristin–dalfopristin	65 (96)	3 (4)	49 (94)	3 (6)
Chloramphenicol	49 (72)	19 (28)	35 (67)	17 (33)
Clindamycin	36 (53)	32 (47)	31 (60)	21 (40)
Penicillin	5 (7)	63 (93)	0 (0)	52 (100)
Linezolid	65 (96)	3 (4)	51 (98)	1 (2)
Minocycline	60 (88)	8 (12)	43 (83)	9 (17)
Cloxacillin	61 (90)	7 (10)	22 (42)	30 (58)
Mupirocin	66 (97)	2 (3)	50 (96)	2 (4)
Rifampicin	64 (94)	4 (6)	49 (94)	3 (6)
Ciprofloxacin	59 (87)	9 (13)	37 (71)	15 (29)

Sabouni et al,[21] highest isolation rate was related to jaundice (12%), and 8% of strains were isolated from wound infection.

In the current study, the highest antibiotic resistance rate was against penicillin (96%), but vancomycin, amikacin, quinupristin–dalfopristin, rifampicin, linezolid, and cefazolin had the least resistance rates. In the study performed by Jung et al,[22] from Korea, similar resistance rates against vancomycin, linezolid, quinupristin–dalfopristin, clindamycin, and rifampicin was found. However, resistance rates of trimethoprim-sulfamethoxazole, tetracycline, and ciprofloxacin were lower than our study. In addition, Cavalcant et al,

from Brazil[23] reported lower rates of resistance for ciprofloxacin, tetracycline, and trimethoprim-sulfamethoxazole. The acquisition of mobile genetic elements (MGEs) harboring multiresistance genes can be a possible reason for higher resistance rates of trimethoprim-sulfamethoxazole, tetracycline, and ciprofloxacin that were observed in this study.[24]

In the present study, HLMR was identified in four isolates (3%). Heterogeneity in mupirocin resistance in different studies has been reported. This rate differs according to the characteristics of studied patients, origin of isolates, and geographical areas.[25] In the study conducted by Pereira

et al, in Brazil,[11] and Emaneini et al, in Iran,[26] none of the tested isolates (0%) were resistant to mupirocin. In the study performed by Hogue et al, in northwest USA,[3] 1.8% of the studied population were colonized with HLMR *S. aureus*. However, in other studies from USA,[9,20] relative high resistance rates were reported (14.7% and 31.3%, respectively). In view of some investigators, there is a strong correlation between the emergence of mupirocin resistance and previous consumption of this drug.[4,8,20,27,28] The low rate of mupirocin resistance observed in this study could be due to the lesser exposure of patients to this antibiotic.

According to the results, 43% (n=52) of our isolates were MRSA which is near to the rate of our previous study (48%)[29] and higher than that reported from Korea (19%),[22] Brazil (26.6%),[23] and Iran (11.5%).[26] The rates of MRSA isolated from blood cultures in our previous study (79%)[30] and the rate reported by McNeil et al from USA (79.5%)[9] were higher than this study. A reason for discrepancies in rates of MRSA could be the diverse source of investigated *S. aureus* isolates in each study and differences in infection control strategies in different regions of the world.[26]

In the current study, there were significant differences in the resistance rates to cefazolin, penicillin, cloxacillin, ciprofloxacin, and amikacin between MRSA and MSSA isolates, and MRSA isolates presented higher rates. There are other reports about higher antibiotic resistance rates of MRSA in comparison with MSSA isolates.[30,31] MRSA isolates possess an MGE called staphylococcal cassette chromosome mec, which carries methicillin resistance gene (ie, *mecA*) and several other genes conferring resistance to non-beta-lactam antibiotics and is in charge of MDR in MRSA isolates.[32] This phenomenon results in the diminished efficacy of most antimicrobial agents in the eradication of MRSA infections and can complicate the selection of proper therapeutic regimens for them.[33,34]

Fortunately, in this survey, the rate of mupirocin resistance in MRSA isolates was low (4%) and was similar to the resistance rate of MSSA isolates (Table 3). However, in the study performed by Baek et al,[33] there were significant differences between the mupirocin resistance rates in MRSA and MSSA isolates (12.7% vs 4.5%, respectively). Furthermore, in the study conducted by Antonov et al,[20] the rate of mupirocin resistance was higher among MRSA isolates and MRSA was recognized as a risk factor for resistance to this antibiotic. In contrary, McNeil et al,[9] observed more mupirocin resistance among MSSA isolates. With regard to the limited therapeutic options for MRSA, continuous monitoring of the emergence

of antibiotic resistance in these pathogens (especially against mupirocin) and ensuring the usefulness of the treatment regimens is also important.[3,11]

The genotypic and phenotypic characteristics of mupirocin resistance were consistent in two HLMR isolates. However, for two other isolates, there were discrepancies between MIC and PCR results. In the study performed by McNeil et al,[9] similar results were observed, and there were two strains with HLMR phenotype which did not harbor *mup*A gene. In contrast, in the study conducted by Wang et al,[1] *mup*A gene was detected in all of the HLMR isolates. In the study performed by Hesami et al,[35] *mup*A gene was detected in ten out of eleven mupirocin-resistant isolates and one isolate possessed *iles*-1 gene, and *mup*B gene was not detected in any of the strains.

In the study of McNeil et al, two mupA-negative isolates were HMR. Although sequencing of both the isolates was performed, no mutations in *ileS-1* gene were observed. Therefore, it is possible that these isolates acquired HMR by another unknown mechanism.[9]

One of the limitations of this study is the lack of information about the prior use of mupirocin by patients. Therefore, the impact of previous consumption of mupirocin on the emergence of resistance against this drug cannot be evaluated. Another limitation of our study is that the presence of *iles*-1 gene (which is responsible for LLMR in isolates of *S. aureus*) and possible mutations in this gene was not detected. The reason for HLMR in the two *mup*A⁻/*mup*B⁻ was unknown. Further studies about the molecular basis of HLMR in the two *mupA*⁻/*mupB*⁻ isolates are continued.

Conclusion

Due to the low resistance rates of MRSA isolates against vancomycin, linezolid, mupirocin, quinupristin–dalfopristin, and rifampicin in this study, it is suggested that these antibiotics should be used in empirical treatment of intricate infections caused by MRSA. With a view to the disparate results of antibiotic susceptibility testing and PCR for designation of mupirocin resistance, it is recommended that determination of mupirocin resistance cannot be done only on the basis of PCR method and this can increase the amount of false-negative results. Despite the low resistance rate to mupirocin (3%) and vancomycin (0%), these drugs should be prescribed cautiously to reserve important drugs in controlling MRSA infections. Regular monitoring of the usage of mupirocin and the rate of its resistance among MRSA isolates is necessary.

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Disclosure

The authors report no conflicts of interest in this work.

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