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Original Article (Pages: 6515-6525)

Prevalence of Nasal Carriage Methicillin-Resistant *Staphylococcus aureus* with *mec*A Gene among Healthy Primary School Boys in North of Iran; A Cross-Sectional Study

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Abstract

Background: Nasal carriage of *Staphylococcus aureus (S. aureus)* has a key role in the epidemiology and pathogenesis of infection. In this study we aimed to investigate the occurrence of the methicillin resistant *Staphylococcus aureus* (MRSA) and *mec*A gene among healthy primary school boys in North of Iran.

Materials and Methods: This cross-sectional study was conducted from January 2017 to July 2017 in Sari city located in the North of Iran. Nasal swabs were taken from 277 healthy primary school boys. *S. aureus* strains were identified according to the standard microbiological procedures and presence of *spa* gene. Agar screen method was used to determine MRSA. All MRSA isolates were examined for the existence of the *mec*A and *spa* gene by using Multiplex Polymerase chain reaction (PCR) method.

Results: The prevalence of nasal carriage of MRSA was 29.24%. The existence of the *mec*A gene among MRSA strains was 49.38%. The rate of resistant isolated to cefoxitin, vancomycin, cefixime, cefalotin, clindamycin, ,cefazolin, co-amoxiclav, amoxicillin, cotrimoxazole and cefalexin antibiotics were 48.14%, 39.50%, 98.76%, 96.29%, 54.32%, 91.35%, 97.53%, 95.06%, 7.40%, and 100%, respectively.

Conclusion: The high rate of Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA), and presence of *mec*A gene, and resistance to critically antibiotics against MRSA is a therapeutic concern and needs to strategies to prevent community spread of *S. aureus*.

Key Words: Children, *mec*A, Methicillin resistant *Staphylococcus aureus*, Nasal.

*Please cite this article as: Rezai Sh, Peyravii Ghadikolaii F, Ahanjan M, Valadan R, Ahangarkani F, Rezai MS, et al. Prevalence of Nasal Carriage Methicillin-Resistant *Staphylococcus aureus* with *mec*A Gene among Healthy Primary School Boys in North of Iran; A Cross-Sectional Study. Int J Pediatr 2017; 5(12): 6515-25. DOI: **10.22038/ijp.2017.26660.2294**

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Received date: Oct.27, 2017; Accepted date: Nov.22, 2017

1- INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a major problem in the healthcare centers across the world (1-5). *Staphylococcus aureus* (*S. aureus*) is the most common bacterial cause of life-threatening infections, including sepsis, deep abscesses, pneumonia, osteomyelitis, and endocarditis (6, 7). *S. aureus* encodes many virulence factors such as the surface Ig-binding protein A. Staphylococcal protein A is specific surface protein which encodes by *spa* gene. The function of *spa* is to capture Immunoglobulin G (IgG) molecules in the inverted orientation and prevent phagocytosis of the bacteria by the host immune system (8). Nasal carriage of *S. aureus* appears to play a key role in the epidemiology and pathogenesis of infection and a reservoir for MRSA. Carriage of *S. aureus* including MRSA is a significant risk factor for nosocomial and community-acquired infections (9).

Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections occur in healthy people who don't have any risk factors for nosocomial infections. (10). The CA-MRSA appears to be less frequently associated with antibiotic resistance in compare with hospital-acquired MRSA (HA-MRSA). The MRSA contain the *mec*A gene which produces a protein that has a low tropism to all beta-lactam antibiotics (β-lactam antibiotics). Resistance to β-lactam antibiotics is attributed mainly to mutations in the *mec*A gene, but other genetic elements may also be considered for the explanation of the mechanism of resistance (11-14).

Screening the nasal carriage isolates of *S. aureus* for antibiotic resistance patterns will provide guidelines for empiric therapy of CA-MRSA (6). The rate of nasal carriage of *S. aureus* strains varying from 16.8% to 90% worldwide (15-17). In Iran the prevalence of nasal carriage of *S. aureus* among hospital staff has varied between 28.2% and 44.5% (16, 18-20). Although several studies have reported the prevalence of MRSA nasal carriage in patients in healthcare settings, this subject has been little investigated in healthy pediatric in North of Iran (21). The aim of the present study was to determination of the prevalence of MRSA, *mec*A gene and in vitro antibiotic susceptibility pattern of MRSA in nasal of healthy primary school boys in north of Iran.

2- MATERIALS AND METHODS

2-1. Study design and populations

This cross-sectional study was conducted from January 2017 to July 2017 in Sari city, located in the North of Iran. The target population was 277 healthy primary school boys between the ages 6-12 years old. Groups of samples (subjects) were selected by using stratified random sampling method. The schools are regarded as a stratifying. The sample size in each stratify was selected proportional to the size of the classes. The sample size was determined to be 277 subjects by using Cochran's formula (with n=3000, α=0.05, P=0.5, d=0.056).

2-2. Ethical considerations

Written informed consent from parents, who on behalf of the children enrolled in the study, was obtained. This study was approved by the ethics committee of Azad University of Qaemshahr branch (378. ID code: 10730548952006).

2-3. Clinical sample collection and identification of bacteria

A sterile moistened swab was inserted into one nostril in turn, to a depth of approximately 1 cm, and rotated five times. The samples were placed into Stuart transport medium and were immediately transported to the microbiology laboratory of Mazandaran University of Medical Sciences. Identification of the bacteria was performed according to the standard

microbiological procedures (morphology, gram stain, catalase test, coagulase test, and mannitol salt agar fermentation) and confirmed by molecular assay (22, 23).

2-4. Antibiotic susceptibility test, isolation of MRSA Strains

Antibiotic susceptibility test was determined by the Kirby–Bauer method according to the Clinical and Laboratory Standards Institute (CLSI) standards (24). Inoculums were diluted to final concentration ($5*10^5$ colony-forming units per milliliter (CFU/ ml), and inoculated into Mueller-Hinton agar. For detection of MRSA strains, oxacillin screen agar was used. Staphylococcus strains were cultured on Muller Hinton agar containing 4% NaCl and 6 milligrams per liter (mg/L) oxacillin and were incubated for 24 hours (3, 25). Antibiotics used in this study were cefoxitin, vancomycin, cefixime, cefalotin, clindamycin, cefazolin, co-amoxiclav, amoxicillin, cotrimoxazole and cefalexin.

2-4-1. Molecular assay

2-4-1-1. DNA extraction

To extract DNA of bacteria the boiling method was performed. Bacterial colonies were inserted in sterile micro tubes that contained 1 milliliter distilled water. Then they were boiled for 5 minutes at 100 Celsius (°C) and were frozen for 5 minutes and again boiled for 5 minutes then centrifuged for 10 minutes at 3,000 revolutions per minute (rpm). The supernatant containing DNA was used as template for PCR amplification (11).

2-4-1-2. Detection of *spa* and *mec*A gene

Multiplex PCR assay was performed to detection *spa* and *mec*A gene. The set of primers and Multiplex Polymerase chain reaction (PCR) amplification conditions are available in **Table.1**. Polymerase chain reaction for amplifying each genes was performed in a final volume of 15 Microliter (µl) including 7.5 µl Master Mix (2x), 0.5 µl from each primers (100 moles/ µl), 2 µl DNA Template and 3.5 µl de-ionized water.

Table -1: The set of primers and Multiplex PCR amplification conditions

Target genes	Primers (5'-3')	Reference	Thermal cycling condition			
spa	F:5’ TAAAGACGATCCTTCGGTGAGC 3’ R:5’CAGCAGTAGTGCCGTTTGCTT 3’	(26)	Step	Time	Temperature	Number of Cycles
			Primary denaturation	1 minutes (min)	95 °C	1
mec A	F:5’ TCCAGATTACAACTTCACAGG3’ R:5’CCACTTCATATCTTGTAACG3’		Denaturation	30 seconds	94°C	37
			Annealing	30 seconds	59°C	
			Extension	1 min	72°C	
			Final extension	7 min	72°C	1

2-4-1-3. Gel electrophoresis

After performing the PCR reaction, electrophoresis of PCR products was carried out in 1.5% agarose gel at 70 voltages for 50 min. Then, results were evaluated under UV light on the UV Trans illuminator.

2-5. Statistical analysis

Data were analyzed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Descriptive cross tabulation and Chi-square test were used; Exact P-values <0.05 were considered as significant.

3- RESULT

From the total of 277 healthy primary school boys between the ages 6-12 years old, nasal carriage of MRSA was seen in 81(29.24%), 95% confidence interval (CI) (23.85%, 34.63%) cases. **Figure.1** shows the age category of students in terms of nasal carrying MRSA. All 81 isolated had *spa* genes. The *mec*A gene found in 40 (49.38%), 95% CI (38.25%, 60.50%) isolates. **Figure.2** that is the illustration of agarose gel shows the strains containing *spa* and *mec*A genes. The relationship between antibiotic resistance and the presence of *mec*A gene is shown in **Table.2**.

The rate of resistant isolated to cefoxitin, vancomycin, cefixime, cefalotin, clindamycin, ,cefazolin, co-amoxiclav, amoxicillin, cotrimoxazole and cefalexin antibiotics were 48.14%, 95% confidence interval [95% CI] (37.03%, 59.26%), 39.50% , 95% CI (26.62%, 50.38%), 98.76%, 95% CI (96.30%, 100%), 96.29% , 95% CI (92.09%, 100%), 54.32%, 95% CI (43.23%, 65.40%), 91.35%, 95% CI (85.10%, 95.60%), 97.53% , 95% CI (94.07%, 100%), 95.06% , 95% CI (90.24%, 99.88%), 7.40%, 95% CI (1.58%, 15.23%), and 100%, respectively.

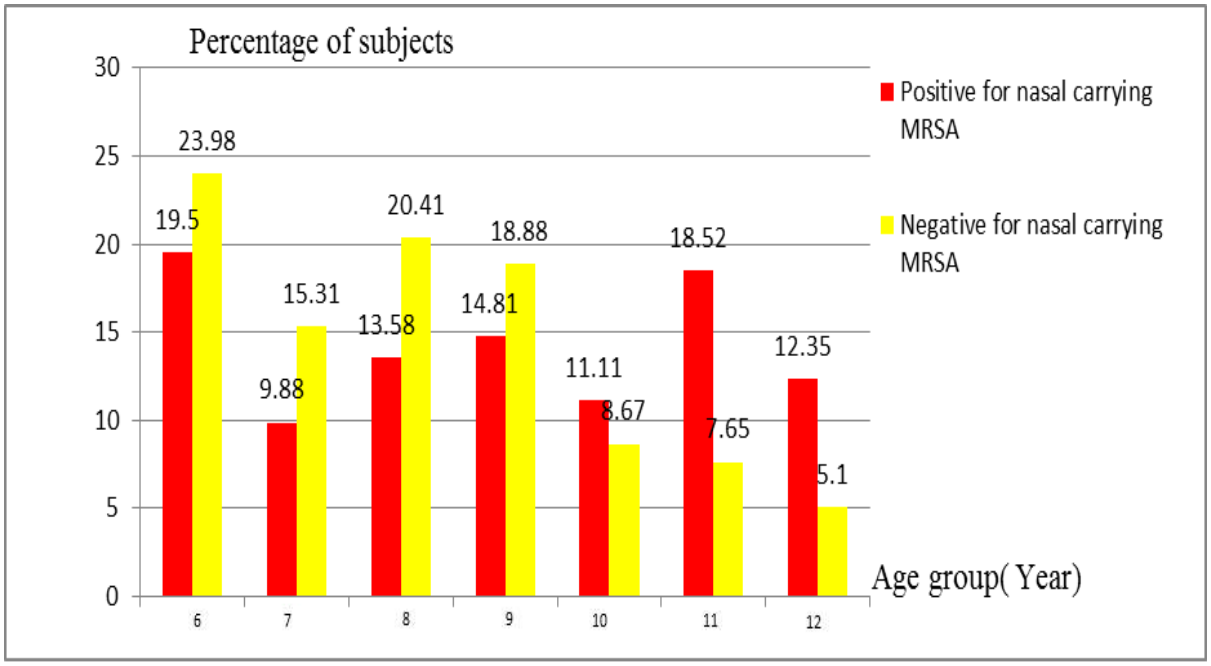

Fig.1: The age category of students in terms of nasal carrying MRSA.

Table- 2: Association between antibiotic resistance and the presence of *mec*A gene in MRSA

Antibiotics	Susceptibility Result	*mec*A		P-value
		Negative	Positive	
		Total number: 41	Total number: 40	
		Number (%)	Number (%)	
cefoxitin	Intermediate	0(0.0)	0(0.0)	.004
	Sensitive	28(68.29)	14(35)	
	Resistant	13(31.70)	26(65)	
vancomycin	Intermediate	25(60.97)	17(42.50)	.218
	Sensitive	2(4.87)	5(12.50)	
	Resistant	14(34.14)	18(45)	
cefixime	Intermediate	1(2.43)	0(0.0)	.999
	Sensitive	0(0.0)	0(0.0)	
	Resistant	40(97.56)	40(100)	
cefalotin	Intermediate	1(2.43)	2(5)	.616
	Sensitive	0(0.0)	0(0.0)	
	Resistant	40(97.56)	38(95)	
clindamycin	Intermediate	4(9.75)	6(15)	.292
	Sensitive	17(41.46)	10(25)	
	Resistant	20(48.78)	24(60)	
cefazolin	Intermediate	2(4.87)	4(10)	.312
	Sensitive	0(0.0)	1(2.50)	
	Resistant	39(95.12)	35(8.5)	
co-amoxiclav	Intermediate	1(2.43)	1(2.50)	.9999
	Sensitive	0(0.0)	0(0.0)	
	Resistant	40(97.56)	39(97.50)	
amoxicillin	Intermediate	0(0.0)	3(7.50)	.177
	Sensitive	1(2.43)	0(0.0)	
	Resistant	40(97.56)	37(92.50)	
cotrimoxazole	Intermediate	37(90.24)	33(82.50)	.214
	Sensitive	3(7.31)	2(5)	
	Resistant	1(2.43)	5(12.50)	
cefalexin	Intermediate	0(0.0)	0(0.0)	-
	Sensitive	0(0.0)	0(0.0)	
	Resistant	41(100)	40(100)	

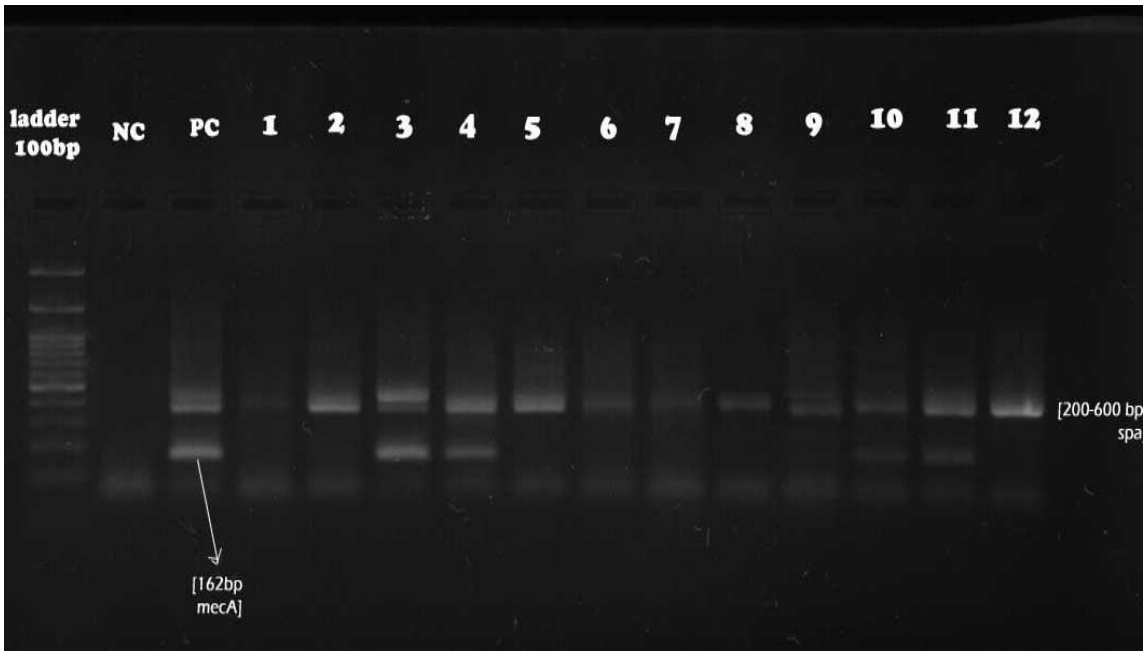

Fig.2: Agarose gel showing the strains containing *spa* and *mec*A genes. The numbers 1 to 12 are the positive strains for *spa* and *mec*A genes. NC: negative control; PC: positive control; bp: base pair.

4- DISCUSSION

The MRSA is one of the major causes of infections worldwide. Increasing resistance against oxacillin in MRSA strains and reducing susceptibility to other antibiotic has posed a huge challenge to treatment of MRSA-related nosocomial infection. In recent years, cases of MRSA infection have been reported in healthy subjects without any exposure to risk factors for MRSA infection. The MRSA carriers in the nose are a major risk factor for infection and transmission of this pathogen (27). In our study, the prevalence of MRSA carriers was 29.24% for boys aged 6 to 12 years, and the rate of resistance to Vancomycin was 45% in these children. Similar to in a study in Hamadan, among 500 children aged 1 to 6 attending day care centers, 26.9% were positive nasal carriage S. aureus and 4.1% were MRSA, contrary to our finding in their study all MRSA were sensitive for vancomycin (28). Tabbarai et al. evaluated 1,193 schoolchildren, 16.3% of children aged 6 to 12 years old were the nasal carrier of *S. aureus* and 34.8% of strains were MRSA; also resistance to vancomycin in these strains was 1.7%. (29); our findings are alarming for the presence of vancomycin-resistant *S. aureus* among healthy children, which should be addressed in a future larger study. In a study on 489 children aged 5 to 15 years old by Chatterjee et al., 52.5% of the children were nasal carriers of *S. aureus*, of which 3.9% were MRSA. The incidence of MRSA in Chatterjee et al. study was lower than our findings (30).

The CA-MRSA isolates are often resistant to fewer classes of antibiotics than HA-MRSA isolates. However, our isolates showed a high resistance to vancomycin (39.5%) clindamycin (54.32%) that are non-β-lactam antibiotics. Our findings are similar to results of Mobasherizadeh et al. that have reported higher resistance rates to non-β-lactam agents among CA-MRSA isolates (31). Clindamycin remains a treatment option of infection caused by MRSA if the clinician is notified of the risk by the microbiology laboratory and the clinical situation is suitable and

vancomycin has been considered to be the reference standard for the treatment of invasive MRSA (32, 33). The high rate of resistance to these critical antibiotics in our study is significant and dangerous. As the aim of our study was focusing on evaluating the extent of CA-MRSA among children rather than an assessment of vancomycin or clindamycin sensitivity, the method of determining the sensitivity in our study was based on antibiogram and minimum inhibitory concentration (MIC) method will be applied in future studies.

The maximum amount of pathogens in the nose can be seen during 2-3 years and in this age range, many germs, such as *Pneumococcus*, *Haemophilus influenzae*, *Moraxella catarrhalis* and *S. aureus*, compete for the colonization of the anterior nasal area (34). Although, there was a significant relationship between age and the incidence of nasal carriage MRSA in our study, so that as the age increased the prevalence of MRSA increased ($p<0.05$). The prevalence of *mec*A gene in MRSA strains in our study was 49.38%. In the meta-analysis of Askari et al., who surveyed the incidence of *mec*A gene in 48 published articles in Iran, of 7,464 *S. aureus* strains, 52.7% ± 4.7 strains had *mec*A gene (35).

In general, the frequency of mecA gene among *S. aureus* has been reported differently in different parts of the world and Iran, so that the prevalence of *mec*A gene in the study by Rezazadeh et al. (2012), 80%, in the study of Diabah et al. (2014), 46.3%, in Udo et al. (2014), 44.3%, and in the study of O 'Malley et al. in (2014), were 42% (36-39). These differences can be due to the different distribution of the gene in various locations or related to the diagnostic methods. But the common thread among all of these studies is the widespread expansion of the *mec*A gene in the world, which indicates a potential risk of the MRSA infections and resistant to a range of other antibiotics in the world. In our study, the presence of *mec*A gene in resistant isolated to cefoxitin, vancomycin, cefixime, cefalotin, clindamycin, clindamycin, cefazolin, co-amoxiclav, amoxicillin, cotrimoxazole, and cefalexin were 65%, 45%, 100%, 95%, 60%, 87%, 97%, 92%, 12%, and 100%, respectively. While in the study of Mahdiun et al., all isolates were resistant to cefoxitin, and after that, the highest and lowest resistance was observed in erythromycin (58.4%), and cotrimoxazole (41.7%), respectively (40). In our study, there was a significant relationship between the presence of *mec*A gene and resistance to cefoxitin ($p <0.05$). However, in the case of other antibiotics, there was no significant relationship between *mec*A gene and antibiotic resistance.

Despite several decades of exposure to the cotrimoxazole, MRSA isolates have retained susceptibility to this antibiotic in different geographical locations (41-43). The low rate of cotrimoxazole resistant isolated in our study could be explained by reducing prescription of this drug in our healthcare setting. For example, Martin et al. described a serial cross-sectional study of resistance to cotrimoxazole among all clinical isolates of *S. aureus* during a 16-year period at United States and found resistance to cotrimoxazole increased from 0% to 48% in *S. aureus* isolates obtained from HIV-infected patients due to extensive use of cotrimoxazole as prophylaxis against *Pneumocystis carinii* pneumonia (44).

In a randomized controlled trial including 252 patients, cotrimoxazole did not achieve non-inferiority to vancomycin in the treatment of severe MRSA infections (45). In recent years the incidence of antibiotic resistance has exponentially increased in north of Iran (1, 4, 46-53). Although in our study cotrimoxazole was one of the most effective antibiotics against MRSA, this antibiotic is

recommended for the treatment of uncomplicated skin and soft tissue infections but not for bacteremia or pneumonia caused by MRSA (45).

4-1. Limitations of the study

We did not evaluate healthy primary school girls in this research.

5- CONCLUSION

This study showed that the prevalence of colonization of MRSA in the nose of healthy primary school boys was relatively high. The health education is necessary in school to prevent the spread of colonization of *S. aureus* among children. Also it is essential to apply strategies to prevent community spread of *S. aureus*. For empiric treatment or/and antibiotic prescription for infection caused by MRSA, physicians need to take into the consideration the antibiotic resistance patterns of the CA-MRSA strains beside resistant pattern of MRSA strain isolated from clinical specimens.

6- CONFLICT OF INTEREST: None.

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