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ORIGINAL ARTICLE

Frequency of methicillin-resistant *Staphylococcus aureus* nasal carriage in healthy children

Roya Nikfar[1], Ahmad Shamsizadeh[1,2]*, Tahereh Ziaei Kajbaf[1], Mohammad Kamali Panah[1], Soheila Khaghani[2], Mina Moghddam[1]

[1]Department of Pediatrics, Aboozar Children's Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
[1]Health Research Institute, Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

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ABSTRACT

Background and Objective: The prevalence of community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) is increasing around the world. It involves healthy people and causes a variety of diseases.
Material and Methods: This cross sectional study was conducted from September 2010 - June 2011 on children less than 14 years of Ahvaz, southwest Iran. The participants were selected with two staged cluster sampling. A sterile cotton nasal swab was used to collect the samples from the 864 participants. MRSA isolates were identifed by catalase and coagulase tests and 1 µg oxacillin disk method. Polymerase chain reaction (PCR) was performed on all the MRSA colonies to detect the *mec*A gene. Data was put in SPSS 16 software and descriptive statistics and chi-square test were used for analysis.
Results: Out of 864 children, 471(54.51%) were male and 393 (45.49%) were female. 235 children (27.1%) had *Staphylococcus aureus* and 11 (1.3%) of all children diagnosed with MRSA. PCR showed that 7 colonies (0.8%) had the *mec*A gene.
Conclusion: The results of this study indicate that MRSA exists in healthy children of Ahvaz. Although the prevalence of CA-MRSA is lower than many other regions, it still needs close attention to prevent its transmission. Further studies are needed to identify the risk factors of CA-MRSA.

Keywords: *Staphylococcus aureus, CA-MRSA, PCR, Children.*

INTRODUCTION

The epidemiology of methicillin resistant *Staphylococcus aureus* (MRSA) is changing around the world. Since 1990, an increase in the prevalence of community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) has been reported worldwide (1-6).

CA-MRSA infections occur in healthy people who don't have any risk factors for nosocomial infections. Its severity varies between a superficial skin infection to severe sepsis (7-9). CA-MRSA appears to be less frequently associated with resistance to non-beta-lactam antibiotics, such as clindamycin and trimetoprim-sulfamethoxazole when compared with hospital-acquired MRSA. A methicillin resistant species must contain the *mec* A gene which produces a protein that has low affinity for the binding of most beta-lactams to its target site (10-12).

*Corresponding author: Ahmad Shamsizadeh
Address: Health Research Institute, Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
Tel: +989161136128
Fax: +986134433715
E-mail: shamsizadeh@ajums.ac.ir, shamsizadeh-a@hotmail.com

Previous studies have well established that carriage of *S. aureus* including MRSA is a significant risk factor for subsequent nosocomial and community-acquired infections (13,14). Multiple studies have shown that more than 80% of these infections are originated from the *S. aureus* nasal colonization and it transmits from there to other parts of the body and to other individuals, directly or indirectly (14-16). These colonies are permanently present in 20% of the population and 60% of the population are involved with them alternately (17). The increasing prevalence of MRSA has not affected all communities equally and different studies from around the world have shown a diverse prevalence of CA-MRSA (3,4,18).

Published data about MRSA colonization in healthy children is limited in Iran (19,20). The purpose of this study was to determine the frequency of MRSA carriage in healthy children in Ahvaz. We also sought to characterize the isolates with regard to the presence of the *mec* A gene.

MATERIALS AND METHODS

This cross sectional study was conducted from September 2010 to June 2011 in Ahvaz city of Iran. The target population was healthy children younger than 14 years of age, who attend in schools and day-care centers. The participants were selected using two-stage cluster sampling and elementary schools and 8 middle schools and 4 day-care centers were chosen from each of 4 educational regions of Ahvaz. One class was selected from each school and all the students of each class and all the children of the selected day-care centers were enrolled.

The sample size was calculated by considering a 95% confidence level, α=0.05 and an expected prevalence of 10% (21). According to this, a sample size of 864 was needed for this study.

Participants who were hospitalized in the past 6 months, used antibiotics within the past month or had a history of a chronic disease such as asthma, diabetes or cystic fibrosis were excluded from the study. The study was approved by the Ethics research committee of Ahvaz Jundishapur University of Medical Sciences (AJUMS) and an informed, written consent was obtained from the parents.

The demographic data were collected from each participant. Also, a sterile cotton swab was inserted into each nostril to collect the specimen. The specimen was placed into Stuart's transport medium (Sharlo, Spain) and delivered to the microbiology laboratory of Abuzar children's hospital. The specimens were cultured on mannitol salt agar media (Merck, Germany) and then were incubated at 35°C for 24 to 48 hours. Colonies with mannitol fermentation (colonies with yellow halo around them) underwent catalase, coagulase and DNase tests (22).

MRSA was determined if inhibitory zone formed around 1 µg oxacillin disk (Mast, UK) on Mueller-Hinton agar (Merck, Germany) (23). The potential MRSA isolates were maintained in trypticase soy broth medium (Merck, Germany) which contained 15% glycerol and stored at -70°C freezer until used for PCR assay.

Antimicrobial susceptibility testing(AST) was performed using agar disk diffusion according to the method explained by CLSI method (24).

The plates were transferred to the Infectious and Tropical Diseases Research Center of AJUMS and polymerase chain reaction (PCR) was used to detect *mec*A gene. DNA was extracted using the boiling method. In this method, bacterial colonies were inserted in micro tubes that contained 1ml distilled water. Then they were boiled for 15 minutes at 100°C and centrifuged for 15 minutes at 3000 rpm. The supernatant containing DNA was used as template for PCR amplification. The purity of the DNA was determined by Eppendorf biophotometer (ratio 260nm /280nm).

The forward mecA1 (5'–GTAGAAATGACTGAACGTCCGATAA–3') and reverse mecA2 (5'–CCAATTCCACATTGTTTCGGTCTAA–3') primers were used for the detection of *mec*A gene.

PCR products were analyzed by electrophoresis on 2% agarose gel and visualized with gel Documentation (24). Positive control (*S. aureus* ATCC 33591) and negative control (*S. aureus* ATCC 29213) was used to ensure the accuracy of the results.

Data was saved in SPSS software (version 16) and descriptive statistics and chi-squared test were used for analysis.

RESULTS

Among the 864 participants, 471 (54.51%) were male and 393 (45.49%) were female. Also, 268 children (31.03%) were in the 1 to 6 years old age group,

, 285 (32.98%) were in the 7 to 10 years old age group and 311 (35.99%) were in the 11 to 14 years old age group.

Out of all the cultures, 235 (27.1%) were colonized with *S. aureus*. As shown in Fig. 1, most of the children involved with *S. aureus* were in the 7 to 10 years old age group.

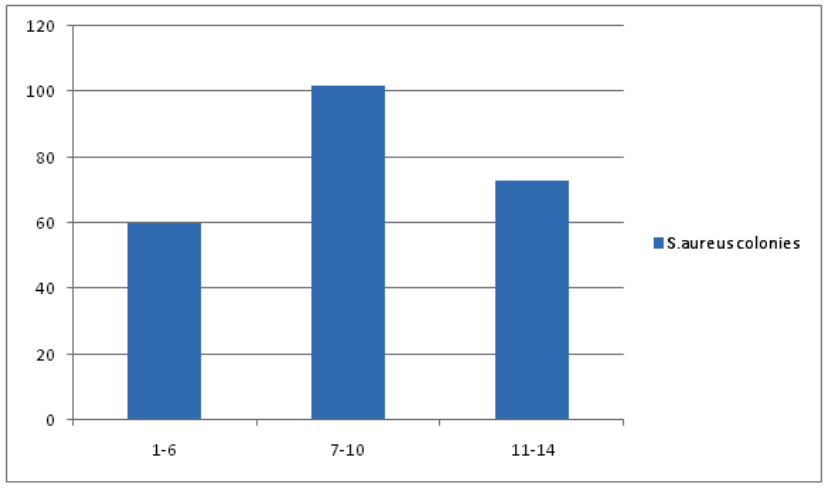

Fig. 1. Frequency of *S. aureus* colonized subjects in each age group

Among the 235 children who were colonized with *S. aureus,* 104 (44%) were female and 131 (56%) were male and 11 cases (4.6%) were carrier for MRSA. Out of them, 7 (63.6%) were female and 4 (36.4%) were male. The difference of the frequency of CA-MRSA among genders was significant (P =0.005). The mean age of the participants with MRSA was 8.09 ± 2.87 years old. All isolates of MRSA were susceptible to vancomycin, clindamycin, gentamicin and trimethoprim-sulfamethoxazole.

The PCR results showed that out of 11 MRSA cases, 7 (63.6% of the MRSA cases and 0.8% of all the participants) were positive for *mec*A gene.

DISCUSSION

This study was conducted to evaluate the prevalence of MRSA nasal colonization in unselected healthy children of Ahvaz city who were aged below 14 years.

In this study, 27.1% of the healthy children were nasal carriers of *S. aureus.* This was consistent with the results of Nakamura (29%) (3), Masuda (28.2%) (25), Sedighi (29.6%)(19). However, the results Sharifi *et al.* (5.2%) (20), and Oguzkaya *et al.* (18%) (26).

We found that most of the children who were involved with nasal *S. aureus* were male (56%). In previous studies it has been demonstrated that rate of colonization with *S. aureus* is higher in male patients than females (25,27,28).

The prevalence of CA-MRSA colonization was 0.8% in healthy children in the present study. Nakamura *et al*. also found 0.8% prevalence for CA-MRSA in the United States (3), Sharifi *et al*. showed 0.5% prevalence in Iran (20), and Suggs *et al*. reported a prevalence of 0.6% for colonization with MRSA (29). On the other hand, other reports show different prevalence. Fritz *et al*. reported 2.4% (30), Sedighi reported 1.2% (19), and Huang *et al*. reported 1.7% prevalence for CA-MRSA (4). It seems that some of the differences of these results are due to the difference of CA-MRSA definition. Salgado *et al*. reported that at least 8 different definitions were used to classify MRSA infections as community acquired, possibly contributing to the heterogeneity among the studies (31).

It should be noted that an increase of proportions of MRSA has been observed over time in some locations. For example in one US center carriage of MRSA increased from 0.8% in 2001 to 9.2% in 2004 (21).

In our study, PCR showed that out of 11 detected MRSAs, only 7 had the *mec*A gene. Many studies have recognized PCR as the gold standard of MRSA diagnosis (18, 32).

S. aureus isolates without *mec*A gene and with methicillin MICs in the 4-16 mg/L range have been reported. These clinical strains termed borderline methicillin resistant *S. aureus* (BRSA) (21).

The major mechanisms believed to BRSA are hyper

production of beta-lactamase. However, these cases were sensitive to amoxicillin/clavulanate. Hussain *et al*. showed that about 1.6% of the *S. aureus* cases with borderline resistant toward methicillin lacked the *mec*A gene (18).

One of the limitations of this study was that we were unable to determine the antibiotic sensitivity of the MRSA cases with the minimal inhibitory concentration (MIC) method.

CONCLUSION

The results of this study indicate that MRSA exists in healthy children of Ahvaz. Although the prevalence of MRSA is lower than many other regions, it still needs close attention to prevent its transmission. Further studies are needed to identify the risk factors of CA-MRSA. Also, according to the likelihood of borderline resistance, determination of the antibiotic sensitivity with the MIC method is recommended.

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