Original Article

Nasal carriage screening of community-associated methicillin resistant *Staphylococcus aureus* in healthy children of a developing country

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Abstract

Background: The rapid emergence and spread of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has raised considerable public health concern in both developed and developing countries. The current study aimed to address the extent of this phenomenon in healthy preschool children of a developing country.

Materials and Methods: We conducted a prospective study from April 2013 to March 2014 on 410 healthy 2-6 years old preschool children in Isfahan, Iran. Demographic medical data and nasal samples were collected from the participating children. Isolates were identified as *S. aureus* and MRSA based on microbiological and molecular tests, including the presence of *eap* and *mecA* genes.

Results: The overall prevalence of *S. aureus* and CA-MRSA nasal carriage was 28% (115/410) and 6.1% (25/410), respectively. The identity of isolates was confirmed by molecular assay. The factors that were independently associated with nasal carriage of *S. aureus* were: Children crowding in day-care nurseries and income level of families. A total of 20/90 (22.2%) of methicillin-susceptible *S. aureus* and all 25 CA-MRSA displayed multiple drug resistance to 3–8 antibiotics.

Conclusions: The current report reflects issues and concerns that the high rate of colonization by CA-MRSA in Iranian healthy children provides obliging evidence that MRSA have established a foothold in the community and are emerging as important health threatening pathogens. It is suggested that we need more effective infection control measures to prevent transmission of nasal CA-MRSA in healthy preschool children.

Key Words: Community acquired, drug resistance, methicillin-resistant *Staphylococcus aureus*, *Staphylococcus aureus*

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INTRODUCTION

Within the past 20 years, methicillin-resistant *Staphylococcus aureus* (MRSA) has been an important cause of nosocomial infection worldwide.[1-5] However, recent studies have documented that the epidemiology of MRSA has changed, as the isolation of MRSA is no longer contained to hospital settings or patients with predisposing risk factors.[4-6] Despite the fact that the prevalence of MRSA colonization in healthy persons in the community is rather low, it has raised concern since it indirectly reflects that there might be a reservoir of people with asymptomatic community-associated MRSA (CA-MRSA) carriage that could act as a source for transmission in the community.[5-7]

In the current study, we aimed to address the extent of this issue in a developing country by evaluating the nasal carriage rates and antimicrobial resistance profiles of *S. aureus* and CA-MRSA in healthy preschool children and its association with established environmental risk factors. Children were selected randomly to represent unbiased population-based prevalence of nasal CA-MRSA carriage.

MATERIALS AND METHODS

Study population and sampling

From April 2013 to March 2014, a total of 410 children attendees between 2 and 6 years of age, who did not have any known risk factors for MRSA colonization were considered eligible to participate in this study. Risk factors that were considered as the excluding criteria were as the following: Hospitalization, surgery, endotracheal intubation, or antimicrobial therapy in the previous 4 weeks, presence of chronic diseases such as asthma, anatomical deformities of nose, and contact with parents or close relatives working in a hospital. They were approached by the same investigative team throughout the study period. The children shared a room in day care centers or kindergartens for an average of 4–5 h in the morning and indoor play areas.

The risk factors analyzed to determine the correlation between them and MRSA colonization included sex, age, and time period of the child attending day care nurseries, city location of the day care center that reflected indirectly the income level of the parents and crowding which was measured by the number of children per 100 m^2 of indoor (primary) space.

DATA COLLECTION

Written questionnaires concerning demographics and medical history (antibiotic usage in the past weeks, duration of antibiotic usage and the name of the antibiotic, presence of a respiratory infection, and having a chronic disease) were completed by the children's parents. Signed informed consent was obtained from the parents. A total of 15 days cares nurseries from five geographical areas, that is, North, West, East, Center, and South of Isfahan were selected by multi-stage sampling. In total, 410 subjects, i.e., around 25–30 from each nursery or kindergarten were recruited for the study.

The study was approved by the ethics committee of Isfahan University of Medical Sciences and the Social Welfare Organization under which the private and public day-care nurseries or kindergartens are organized and operated.

Microbiological method

A specimen for culture was obtained from both anterior nares of each enrolled child with a sterile dry cotton swab, premoistened with sterile water. The swab was immediately inoculated into tryptic soy broth + NaCl 6.5% (Oxoid, UK) and incubated for 24 h at 35°C. They were then subcultured onto mannitol salt agar (Oxoid, UK) using calibrated microbiological loop and incubated at 35°C for 24 h.

Isolates were identified as *S. aureus* based on morphological and biochemical conventional tests including gram stain, catalase, free coagulase, clumping factor, mannitol fermentation, and novobiocin susceptibility.

MRSA were identified by assessment of cefoxitin susceptibility (30 μg disk (Mast, UK) using disc diffusion method and E-test for oxacillin (bioMe´rieux) according to the Clinical Laboratory Standards Institution (CLSI).[8]

Antimicrobial susceptibility test

Antimicrobial resistance profile of the isolates were determined using a panel of 12 antibiotic discs by Kirby–Bauer and E-test methods according to CLSI guideline.[8] The antibiotics included penicillin 10 units, cefoxitin 30 μg, gentamicin 10 μg, ciprofloxacin 5 μg, tetracycline 30 μg, rifampin 5 μg, linezolid 30 μg, clindamycin 2 μg, erythromycin 15 μg, vancomycin 30 μg, and trimethoprim/sulfamethoxazole (co-trimoxazole) 1.25/23.75 μg.

For all isolates, D-test was carried out. *S. aureus* ATCC 25923 and MRSA 33591 were used as quality control strains. Borderline oxacillin (2–3 μg) was scored as resistance.

DNA extraction

The genomic DNA was extracted by simple boiling method.[9] 50 mg of bacterial biomass was suspended

in 400 µl of TES (50 mM Tris hydrochloride [pH 8.0], 5 mM EDTA, 50 mM NaCl), and the suspension was heated at 95°C for 7 min and centrifuged at 10000 g for 10 min. The resultant supernatant was taken as DNA lysate and kept at –20°C for the polymerase chain reaction (PCR) reaction.[9]

Specific identification of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* detection by polymerase chain reaction

The presence of the Eap-encoding (*eap*) and mecA-encoding (*mecA*) genes was assessed by specific PCR amplification for molecular species specific identification and MRSA detection of *S. aureus* isolates, respectively, as recommended in previous studies.[10,11]

In brief, 5 µl of the DNA lysate was transferred with filter-plugged pipette tips to 20 µl of PCR amplification mix. The PCR for molecular identification of S. aureus and detection of MRSA was performed essentially as described previously.[10,11] The PCR cycling conditions were as follows: Initial denaturation at 95°C for 5 min, followed by 30 cycles of 60 s at 95°C, 30 s at 50°C, and 120 s at 72°C, with a 10 min final extension step at 72°C. The PCR products were run on 1% agarose gel, visualized and photographed under UV Transilluminator.

Statistical methods

Statistical Package for the Social Sciences (SPSS) for Windows (version 22; SPSS, Chicago, IL, USA) software was used for the statistical analysis of the data. Frequency and percentage were presented for categorical data. Fisher's exact test was applied to determine potential factors associated with *S. aureus* nasal carriage. The level of significance was set at 0.05 using the two-tailed method. The WHONET 5.6 software (WHO, Switzerland, Geneva) was applied to antimicrobial susceptibility profile analysis.

RESULTS

In the study, 410 children were enrolled from 15 preschools. Out of them, 50.7% (n = 208) were boys and the remaining 49.3% were girls. Most children (82.2%, n = 334) belonged to the age group of 5–6 years of age [Table 1].

Out of 410 children included in the study, 115 children were culture positive for *S. aureus*. The identification was confirmed by typical microbiologic tests including gram positive cocci and positive tests for catalase, free coagulase, clumping factor, mannitol fermentation, and novobiocin susceptibility. Out of 115 *S. aureus* isolates, 25 were identified as MRSA by assessment of cefoxitin susceptibility. 5 out of 25 MRSA isolates showed borderline resistance to oxacillin (minimum inhibitory concentration =3 µg/mL). The identity of *S. aureus* and MRSA isolates were confirmed by PCR detection of the *eap* and *mecA* genes, respectively [Figure 1].

The prevalence of *S. aureus* nasal carriage was 28% (115/410) (95% confidence interval [CI]: 25.95–29.46) and that of MRSA was 6.1% (25/410) (95% CI: 5.67–6.45). This figure corresponds to 21.7% (25/115) of *S. aureus* isolates (95% CI: 20.57–22.83).

The factors that were independently associated with nasal carriage of *S. aureus* were: Children crowding in day-care nurseries; $P < 0.01$, income level of families ranking as low, middle, and high income families, $P < 0.005$, however, the factors which were not associated with nasal carriage were age-group; $P = 0.41$, sex; $P = 0.5$ and time period of child attending day care nurseries; $P = 0.55$ [Table 1].

All *S. aureus* isolates in our study were β-lactamase positive. The antibiotic susceptibility pattern of methicillin-susceptible *Staphylococcus aureus* (MSSA) and MRSA to individual antibiotics is showed in Table 2 and Figure 2.

The co-resistance patterns noted among the Iranian isolates suggest that 4% (1/25), 20% (5/25), 8% (2/25), and 16% (4/25) of CA-MRSA and 1.1% (1/90), 2.2% (2/90), and 17.8% (16/90) of CA-MSSA were resistant to 6, 5, 4, and 3 non-β-lactam antimicrobial groups, i.e., ciprofloxacin, clindamycin, erythromycin, gentamicin, tetracycline, and cotrimoxazole, while 77.8% (70/90) of CA-MSSA isolates were resistance to less than three non-β-lactam antibiotics.

D-test for macrolide-inducible resistance to clindamycin was positive for 4/25 (16%) of MRSA isolates and 11/90 (12.1%) of MSSA isolates ($P = 0.99$).

DISCUSSION

After nearly three decades of being solely confined to hospitals and long-term care facilities, MRSA has emerged in various geographically distinct communities outside of health care settings, without known health care-associated risk factors.[12,13] Despite the straight forward epidemiology of MSSA, there are only a few reports on epidemic MRSA.[13,14] Much of the published literature related to the epidemiology of MRSA in day care centers has focused on outbreaks.[15] These studies were reviewed elsewhere.[16] Such studies have studied the source, transmission pathways, and control measures to stop

Table 1: Frequency distribution of *S. aureus* and CA-MRSA nasal carriage based on demographic variables

Variable	*n* (%)	*S. aureus* nasal carriage (%)		*P*	CA-MRSA nasal carriage (%)		*P*
		Negative	Positive		Negative	Positive	
Age group (years)							
2	13 (3.2)	11 (2.7)	2 (0.5)	0.47	13 (3.2)	0 (0)	0.41
3	19 (4.6)	12 (2.9)	7 (1.7)		19 (4.6)	0 (0)	
4	41 (10)	31 (7.6)	10 (2.4)		39 (9.5)	2 (0.5)	
5	123 (30)	94 (22.9)	29 (7.1)		119 (29)	4 (1)	
6	214 (52.2)	147 (35.9)	67 (16.3)		195 (47.6)	19 (4.6)	
Sex							
Male	208 (50.7)	150 (36.6)	58 (14.2)		195 (47.6)	13 (3.2)	0.5
Female	202 (49.3)	145 (35.3)	57 (13.9)		190 (46.3)	12 (2.9)	
The time period of child attending day care nurseries (years)							
1	264 (64.4)	195 (47.6)	69 (16.8)	0.14	242 (59.1)	22 (5.4)	0.55
2	68 (16.6)	50 (12.2)	18 (4.4)		66 (16.1)	2 (0.5)	
3	52 (12.7)	29 (7.1)	23 (5.6)		51 (12.4)	1 (0.2)	
4	16 (3.9)	12 (2.9)	4 (1)		16 (3.9)	0 (0)	
5	10 (2.4)	9 (2.2)	1 (0.2)		10 (2.4)	0 (0)	
Residential area in Isfahan							
North	105 (25.6)	69 (16.8)	36 (8.8)	0.2	95 (23.2)	10 (2.4)	<0.005
Western	81 (19.8)	65 (15.9)	16 (3.9)		81 (19.8)	0 (0)	
Eastern	96 (23.4)	67 (16.3)	29 (7.1)		83 (20.2)	13 (3.2)	
Southern	64 (15.6)	47 (11.5)	17 (4.1)		62 (15.1)	2 (0.5)	
Center	64 (15.6)	47 (11.5)	17 (4.1)		64 (15.6)	0 (0)	
Crowding (per 100 m^2 of indoor space)	-	30	33	0.37	31	43	<0.01

CA-MRSA: Community-associated methicillin-resistant *S. aureus*, *S. aureus*: *Staphylococcus aureus*

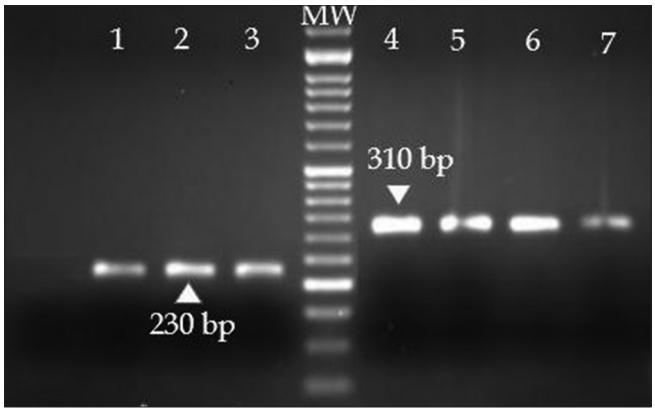

Figure 1: Polymerase chain reaction assay for detection of *eap* and *mecA* genes. Lanes: 1 and 4: Reference international strains of *Staphylococcus aureus* ATCC 25923 and methicillin-resistant *Staphylococcus aureus* ATCC 33591 Lanes: 2, 3, 4, 5, 6, and 7, representative methicillin-resistant *Staphylococcus aureus* strains isolated in the current study; MW, 50-bp DNA ladder marker

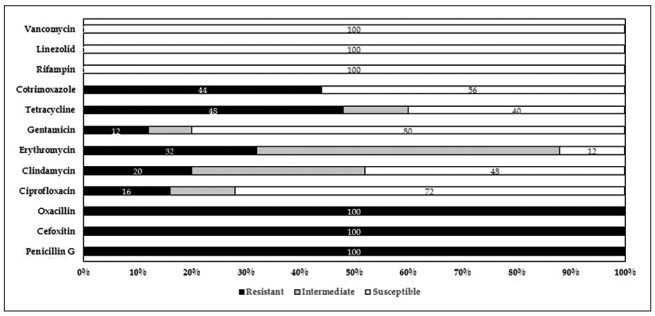

Figure 2: Antimicrobial susceptibility profiles of Iranian community-associated methicillin-resistant *Staphylococcus aureus* isolates based on disk diffusion and E-test methods

further spread of infection. Most people with MRSA have a history of recent hospitalization, surgery or dialysis, residence in a long-term care facility or an implanted medical device.[17-20]

Since the late 1990s, a number of studies have demonstrated that MRSA colonization and infection seen among healthy babies, toddlers, children, and adults who do not have these healthcare-associated risk factors. It appears that MRSA in the community has a complex epidemiology originating from the dissemination of feral descendants of hospital isolates HA-MRSA strains in the general population or arising from horizontal transfer of the methicillin-resistance determinant into a formerly susceptible background.[21-24] Colonization may be transient or persistent and can last for years.[25]

Having these facts in mind, the current study designed to evaluate the extent of this issue in a developing country by estimation of the prevalence of nasal carriage and antimicrobial resistance profiles of *S. aureus* and CA-MRSA in healthy preschool children.

Table 2: Antimicrobial susceptibility profiles of CA-MSSA and CA-MRSA based on disk diffusion and E-test methods

Antibiotics	Strain	R[a]	I[b] (%)	S[c]	*P*	MIC range (μg/mL)
Penicillin G	CA-MRSA	100	0	0	0.99	-
	CA-MSSA	100	0	0		-
Cefoxitin	CA-MRSA	100	0	0	<0.001	-
	CA-MSSA	0	0	100		-
Ciprofloxacin	CA-MRSA	16	12	72	<0.01	-
	CA-MSSA	4.4	10.3	85.3		-
Clindamycin	CA-MRSA	20	32	48	<0.9	-
	CA-MSSA	22.2	32.2	45.6		-
Erythromycin	CA-MRSA	32	56	12	<0.05	-
	CA-MSSA	23.6	50.6	25.8		-
Gentamicin	CA-MRSA	12	8	80	<0.05	-
	CA-MSSA	3.3	3.3	93.3		-
Tetracycline	CA-MRSA	48	12	40	<0.01	-
	CA-MSSA	26.2	9.5	64.3		-
Cotrimoxazole	CA-MRSA	44	0	56	<0.003	-
	CA-MSSA	6.2	2	91.8		-
Rifampin	CA-MRSA	0	0	100	0.99	-
	CA-MSSA	0	0	100		-
Linezolid	CA-MRSA	0	0	100	0.99	-
	CA-MSSA	0	0	100		-
Oxacillin	CA-MRSA	100	0	0	<0.001	3-48
	CA-MSSA	0	0	100		0.125-1.5
Vancomycin	CA-MRSA	0	0	100	0.9	0.25-2
	CA-MSSA	0	0	100		0.125-2

[a]R: Resistant, [b]I: Intermediate, [c]S: Susceptible, CA-MRSA: Community-associated methicillin-resistant *S. aureus*, CA-MSSA: Methicillin-susceptible *S. aureus, S. aureus: Staphylococcus aureus*, MIC: Minimum inhibitory concentration

The overall *S. aureus* prevalence among healthy children participating in this study was 28%. Our prevalence (6.1%) was almost three times higher than an overall prevalence of 2.7% for MRSA colonization reported based on a recent meta-analysis of studies conducted on four different continents.[20] An almost similar rate 6.2% of *S. aureus* colonization has been reported from Brazil.[26] However, the rate of CA-MRSA carriage of 6.1% in this study is not within the reported range for children from some countries. A higher MRSA colonization prevalence rate was found in our study in comparison with a previous study from West part of Iran, Hamedan, (6.1 vs. 4.1%),[27] as well as that of reported in similar studies from countries such as Taiwan 7.3% (2), Colombia 4.8%,[28] and Turkey 5.6%.[17] The prevalence rate of MRSA colonization for Iranian children found in our study was lower than that of reported for children from South Korea (9.3%)[29] in 2008, India 10.2%[30] and in Taiwan 13.2%.[7]

All *S. aureus* and MRSA isolates were classified as CA-MRSA because the children had not undergone surgery or hospitalization in the previous 12 months. When coupled with the absence of an association between colonization a known risk factor, the high prevalence of MRSA and *S. aureus* in day care centers suggests that these settings may serve as reservoirs for CA-MRSA colonization.

CA-MRSA isolates are often resistant to fewer classes of antimicrobial agents than HA- MRSA isolates.[4,31,32] However, our isolates showed a high resistance to non-β-lactam antibiotics, i.e., tetracycline (48%), cotrimoxazole (44%), erythromycin (32%), clindamycin (20%), ciprofloxacin (16%), and gentamicin (12%). This finding is consistent with the reports from Asian countries that have demonstrated higher resistance rates to non-β-lactam agents among CA-MRSA isolates than that of reported for isolates from American or European countries, therefore making difficult to distinguish them from HA-MRSA by antibiogram only.[7,29,30]

Crowding has been linked to a number of biological mechanisms that can increase both the risk and the intensity of infection.[33] In our study, it was observed that when the number of attending children in a day care center increased, the prevalence of nasal carriage of CA-MRSA increased as well. Similarly, some investigators reported that MRSA colonization was associated with the number of children in the day care center.[34-36] Association of attending children with nasal carriage of *S. aureus* is probably due to overcrowding and greater sharing of nasal flora within a day care center.

Income inequality is highly correlated with *S. aureus* methicillin resistance.[27] This phenomenon reveals itself in our study with a higher CA-MRSA prevalence in day care centers located in a poorer neighborhood (Northern and Eastern parts) where children come from poorer families.

Both extent and pattern of antibiotic utilization are important determinants of antibiotic resistance. In our country, antibiotic use is quite high either in the hospitalized patients or outpatients where antibiotics are freely available for purchase over the counter. Strict monitoring and regulation are urgently required to promote wise use of antibiotics.

CONCLUSIONS

The high rate of nasal carriage of *S. aureus* and CA-MRSA and presence of resistance to commonly used antibiotics is a big concern. Antibiotic surveillance program and infection control strategy should be instituted to monitor the development of antibiotic resistance nationally through clear and feasible implementation strategies to prevent community spread of resistant bacteria.

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Conflicts of interest

There are no conflicts of interest.

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