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Comparison of Mec A Gene in Staphylococcus Aureus Isolated from Children at Time of Admission and Discharge in West of Iran

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ABSTRACT

Staphylococcus aureus (SA) is a species of the genus Staphylococcus that is found everywhere, including in the respiratory tract and on the skin of many adults and children, and that is considered one of the main pathogens in nosocomial and community acquired infections. The number of Methicillin-Resistant Staphylococcus aureus (MRSA) strains has increased globally over the past two decades. Determining the frequency of mecA gene in SA that represent MRSA in hospitalized children and comparing it upon hospital admission and discharge therefore appear essential. The present cross sectional study was conducted in children hospitalized in Imam Reza hospital in Kermanshah, Iran in 2012. Nasal specimens were collected from the 500 children at time of admission, considered as community acquired Staph aureus and those whose first nasal culture was negative were included for second culture at time of discharge, considered as hospital acquired Staph aureus. After identifying Staphylococcus aureus by common laboratory tests, methicillin resistance was determined using Oxacillin screening plates and mec -A gene. The prevalence of community acquired Staph aureus nasal carriers was estimated as 4.6% and the prevalence of its hospital-acquired type as 5.6%. The prevalence of mecA gene was 17/4% and 37% in two groups, suggesting no statistically significant differences between the two groups. The resistance to Erythromycin, Cefoxitin, Mupirocin and Clindamycin was 30/4%, 8/7%, 4/3% and 30/4% in the admission group and 40/70%, 7/4%, 7/4% and 18/5% in the discharge group, suggesting no statistically significant differences between the two groups. All the samples were sensitive to Vancomycin and Linezolid and no instances of resistance to these antibiotics were observed. The increase in the prevalence of MRSA is caused by the overuse of antibiotics, which facilitates the colonization of MRSA. Moreover, the increase in the resistance to commonly-used antibiotics is a warning for a more reasonable prescription of sensitive antibiotics such as Vancomycin, so as to prevent the emergence of multidrug resistant MRSA.

Keywords: Staphylococcus aureus, hospital, Multiplex PCR

INTRODUCTION

After the first strains of Methicillin-resistant Staphylococcus aureus [MRSA] were detected in 1961, a rapid increase was observed in the prevalence of nosocomial MRSA infections and their associated mortality rates. By definition, Methicillin-resistant Staphylococcus aureus are strains of Staphylococcus aureuses that either carry mec A gene or exhibit a minimum inhibitory concentration of 4 µg/ml to oxacillin [1]. Resistance to Methicillin and other derivatives of the β-lactam family of antibiotics is caused by the presence of mec A genes with a length of 2.1 kb [3]. In spite of the presence of mecA gene, MRSA strains may have different MICs; in fact, strains with an MIC of 4-8 µg/ml and no mecA gene are not rare. PCR is therefore considered the best standard method of identifying

mecA gene [4]. Staphylococcus aureus have either become or are becoming resistant to all known types of practical antibiotics such as β-lactamse, Glycopeptides, Aminoglycosides, Quinolons, etc. [1]. In the past, Methicillin-resistant Staphylococcus aureus strains or the so-called hospital-acquired MRSA [HA-MRSA] were limited to hospitals [3]. With the advent of Methicillin-resistant strains in the community, the resistance to Methicillin became a critical issue. The so-called community-associated MRSA [CA-MRSA] strains are resistant to Methicillin; however, their pattern of drug sensitivity to antibiotics other than β-lactams is similar to in Methicillin-sensitive Staphylococcus aureus [MSSA] strains [4]. Furthermore, unlike HA-MRSA, these strains do not require underlying risk factors such as hospitalization, chronic diseases, renal dialysis, drug use or HIV infection [5]. CA-MRSA stains are often cause mild infections of the skin and soft tissues; however, severe and life-threatening cases such as necrotizing pneumonia, necrotizing fasciitis, myonecrosis and sepsis are also emerging [6]. The prevalence of MRSA, which has increased in the recent decade, is an important factor for increase in treatment costs and hospital mortality rates. MRSA infections are currently responsible for about 19,000 hospital mortalities in the US, which is equal to the total number of deaths caused by HIV, tuberculosis and viral hepatitis in the US [8]. The prevalence of MRSA varies in different countries and even from one hospital to another [7]. The strains are more problematic in closed communities such as barracks, kindergartens, prisons and similar institutions in which people are in close contact with each other for prolonged periods of time [9]. Infection with Methicillin-resistant Staphylococcus aureus circulating in the community has dangerous consequences, especially since there are no effective medicinal therapies to treat the infection. Given the importance of CA-MRSA and the need for the evaluation of its prevalence across the community and the lack of adequate information available on the subject in Iran, the present study was conducted to give a more accurate estimation of the current prevalence of MRSA in the community and in the age group that is less contact with hospital settings.

MATERIALS AND METHODS

The present study was conducted on 500 children hospitalized in Imam Reza hospital in Kermanshah selected through census sampling in 2012. Nasal swab screening was performed on the samples upon hospital admission and discharge. The samples cultured in specific media were isolated and their strains were detected. Oxacillin screening plates were used according to the Clinical and Laboratory Standards Institute [CLSI] guidelines so as to determine the resistance to Methicillin. Patients with a history of antibiotic use one week or corticosteroid use two weeks before hospitalization, immunodeficiency, a history of hospitalization one month before presenting to the hospital, those with chronic systemic diseases such as diabetes, renal failure or Staphylococcus aureus infection were excluded from the study and all the other hospitalized patients were included in the study. If the results of samples of patients that were included at time of admission were positive they were considered as community acquired staph aureus [CASA]. They were excluded from sampling at time of discharge. If the result of samples were negative at time of admission but positive at discharge time, it considered as hospital acquired staph aureus[HASA]. For the sampling stage, a cotton swab soaked in a sterile saline solution was pushed into the patients' anterior nostrils and rotated five times in each. The swabs were cultured on Mannitol Salt Agar media on the site of sampling and were then transferred to the School of Medicine within a maximum of two hours and were incubated at 35º C for 24-48 hours. Yellow colonies [Mannitol yeast] suspected of Staphylococcus aureus were cultured on blood agar medium for subsequent tests. Colony morphology, gram staining, catalase test, slide coagulase test, tube coagulase test and DNase test used to identify the bacterium. Isolates of Staphylococcus aureus were inserted into culture media with the minimum number of subculture and were then kept in the fridge until PCR and antibiotic susceptibility test were performed.

DNA Extraction

DNA extraction was performed by boiling method. The bacteria kept in the freezer were cultured on nutrient agar plates and incubated at 37º C for 18-24 hours. A loop was removed from the first culture zone and dissolved in 250 µL of sterile injectable distilled water and the bacterial suspension was then incubated for 10 minutes at 90º C (in a 90º C bain-marie). The suspension was centrifuged at 7500 rpm for 5 minutes. The solution containing the DNA was transferred to an empty micro tube.

Determining the DNA concentration

The DNA sample was diluted 1:100 and a spectrophotometer was used to read its optical absorption at wavelengths of 280 and 260 nm. The first wavelength shows the OD of the protein and the second wavelength the OD of the DNA. The OD read at 280 nm is used to calculate the OD 260: OD 280 ratio, as the presence of protein indicates impurity in the extracted DNA. The closer is the ratio to 1:8; the higher is the DNA purity.

PCR gene identification

After extracting the DNA from the bacteria, PCR is performed through the following method:

A total of 5 µL of the boiled DNA extract, 0.5 µmol of the primer, 1.5 mmol of MgCl2, 0.2 mmol of dNTP and 0.25 unit of Taq enzyme were used. The thermal cycle used involved:

45 seconds at 94º C
30 cycles of 20 seconds at 94º C
15 seconds at 57º C
15 seconds at 70º C
2 minutes at 72º C

Identification of the PCR product
A horizontal electrophoresis system and agarose gel was used to identify the molecular weight of the PCR product. The 1x buffer TBE used was diluted from stock solution. A 1.5% agarose gel was prepared from dissolving 0.3 gr of agarose in 20 mL of TBE 1x buffer. After dissolving the agarose over low heat and allowing it to cool down and reach an approximate temperature of 40-50º C, it was cast on gel support frame with comb in place. After the gel was solidified, the comb was removed and the gel was placed in an electrophoresis chamber containing TBE 1x buffer. The comb and the tray were washed in advance. The chamber was then connected to a power supply device. The negative poll of the device was connected to the wells. The device was first set on 60 V/20 min and then on 45 V/70 min. The DNA was poured into the wells with a loading buffer solution.

To detect DNA segments under UV light, an Ethidium Bromide solution with a final concentration of 0.05 µg/ml was used, which can be poured directly into the gel before it is solidified or the gel can be poured into an Ethidium Bromide solution with the same concentration after electrophoresis is complete and can then be taken out of the solution within a few minutes to be seen under UV light. Ethidium Bromide solution is available as a 10 mg/µl stock solution. Safety precautions should be observed when working with Ethidium Bromide and the skin should not touch the substance. A Gel Doc was used for imaging the gel. The antibiotic susceptibility test was then performed with a number of antibiotics including Linezolid, Cefoxitin, Erythromycin, Clindamycin, Vancomycin and Mupirocin using the Kirby-Bauer method (21). Oxacillin disks were used for all the Staphylococcus aureus samples. The results obtained were then investigated to determine the degree of colonization by SA in the community and in the hospital and to compare patients in terms of the presence of mecA gene. The data collected were analyzed in SPSS using the Chi-square test and Fisher's exact test at a significance level of $P<0.05$.

RESULTS

Nasal swabs were taken from 500 hospitalized children upon their hospital admission and discharge. The children with positive results upon admission were referred to as cases of community-acquired Staphylococcus aureus (CASA) and those with negative result at time of admission but positive results upon discharge considered as cases of hospital-acquired Staphylococcus aureus (HASA).when a case of CASA or HASA was resistant to oxacillin or had mec A gene considered as CA-MRSA and HA-MRSA respectively. A total of 61 samples were infected with Staphylococcus aureus, 23 of which were Staphylococcus aureus positive upon admission and 38 only upon discharge. Of the 38 cases that were positive at discharge time, 11 were also positive upon admission and were thus classified as CASA and deducted from the 38 cases. The remaining 27 Staphylococcus aureus positive samples from the total of 477 children without positive results upon admission were classified as HASA. The present study estimated the prevalence of nasal carriers of CA-MRSA as 4.6% and the prevalence of nasal carriers of HA-MRSA as 5.6%. Oxacillin resistance was observed in 4 of the samples who were SA-positive upon admission and 10 of those who turned out SA-positive upon discharge, all of whom carried mecA gene (MRSA). There were no differences between the results obtained through the Oxacillin disks and the PCR. A total of 4 of the samples that were SA-positive upon admission carried mecA gene, constituting a prevalence of 0.8% of MRSA in them (the prevalence of CA-MRSA). As for the samples that turned out SA-positive upon discharge and negative at admission time, 10 carried mecA gene, constituting a prevalence of 2% (the prevalence of HA-MRSA). The mean age of participants was 56±48/7 in the CASA samples and 36/15±29/9in the HASA samples, suggesting the lack of significant differences between the two groups. Although most cases were male but no meaningful different between them were observed. The prevalence of mec A gene was 17/4% in the CA-MRSA and 37% in the HA-MRSA group, showing the lack of a statistically significant difference between the two groups. The resistance to Erythromycin, Cefoxitin, Mupirocin and Clindamycin was 30/4% and 8/7%, 4/3% and 30/4% in the CA-MRSA group and 40/7%, 7/4%, 7/4% and 18/5% in the HA-MRSA group, suggesting no statistically significant differences between the two groups. All the samples were sensitive to Vancomycin and Linezolid antibiotics and no instances of resistance were observed.

Table 1: The mean age of the patients in the CA and HA groups

	Number	Mean	Standard Deviation	P-Value
Community	23	56.00	48.735	0.98
Hospital	27	36.15	29.915	

Table 2: The frequency distribution and relative frequency of the gender variable in the CA and HA groups

Gender	Group		P-Value
	Community	Hospital	0.555
Female	10 (43.5%)	14 (51.9%)	
Male	13 (56.5%)	13 (48.1%)	

Table 3: The frequency distribution and relative frequency of the mecA gene variable in the CA and HA groups

mecA	Group		Total	P-Value
	Community	Hospital		0.123
Negative	19 (82.6%)	17 (63.0%)	36 (72.0%)	
Positive	4 (17.4%)	10 (37.0%)	14 (28.0%)	

Table 4: The frequency distribution and relative frequency of antibiotic resistance in the CA and HA groups

		Community	Hospital	P-Value
Erythromycin	Sensitive	16 (69.9%)	16 (59.3%)	0.449
	Resistant	7 (30.4%)	11 (40.7%)	
Cefoxitin	Sensitive	21(91.3%)	25(92.6%)	1.00
	Resistant	2(8.7%)	2(7.4%)	
Mupirocin	Sensitive	22(95.7%)	25(92.6%)	1.00
	Resistant	1(4.3%)	2(7.4%)	
Clindamycin	Sensitive	16(69.6%)	22(81.5%)	0.325
	Resistant	7(30.4%)	5(18.5%)	

DISCUSSION

The early diagnosis and treatment of MRSA infections are crucial measures for preventing the spread of the infection and reducing the mortality risks associated with it. In addition to Methicillin and β-lactam, MRSA strains are even more resistant to other antibiotics [10-13]. The present study reported the prevalence of Staphylococcus aureus nasal carriers as 4.6% in the community and 5.6% in the hospital group and the prevalence of mecA gene as 17/4% and 37% in CASA and HASA respectively. A study conducted by Moghadami et al. [2010] investigated isolates of SA from CA-MRSA and HA-MRSA samples in terms of their drug and epidemiologic resistance. The isolates were collected from seven hospitals and about 14% were MRSA [14]. The results of the present study showed a 37% prevalence of mecA gene in hospital acquired Staphylococcus aureus strains, which is similar to the prevalence of the strains in large hospitals in Southeast Asia [15 and 16] and less than in hospitals in northern Europe and the Middle East [except for large hospitals in Saudi Arabia]; [17-21]. A study conducted by Fan reported the prevalence of CA-MRSA as 18% [22]. In a study by Wen, the prevalence of Staphylococcus aureus nasal carriers decreased from 28% in 2004-2006 to 23% in 2007-2009, while the prevalence of MRSA identified by mecA gene PCR increased from 8% to 15/1% during the same period [23], which is in line with the results of the present study. The results reveal that, in spite of the reduction in the prevalence of community-associated Staphylococcus nasal carriers, the prevalence of CA-MRSA has increased in proportion to it, which could be due to the overuse of antibiotics that eradicate Methicillin-sensitive Staphylococcus and facilitate MRSA colonization. In a study conducted in Kashan, 150 Staphylococcus aureus samples isolated from clinical [urine, blood, etc.] specimens were analyzed by PCR, 87 [58%] of which were found to be carriers of mecA gene, with 34 [39%] of the 87 strains being hospital-acquired and 53 [60.9%] community-associated. Half of the strains carried mecA gene while more than 60% were community-associated [CA-MRSA]; [24]. The results of this study are not consistent with the findings of the previous study, perhaps due to the exclusion of children with underlying diseases, who might themselves be one of the major carriers of community-associated Staphylococcus aureus. The resistance to Erythromycin, Cefoxitin, Mupirocin and Clindamycin was calculated as 30/4%, 8/7%, 4/3% and 30/4% in the CASA group and as 40/7%, 7/4%, 7/4% and 18/5% in the HASA group. While all the groups were sensitive to Vancomycin and Linezolid, the resistance to Erythromycin, Clindamycin and Mupirocin also increased. No instances of resistance to Vancomycin and Linezolid were observed. Alborzi et al. examined 156 cases of MRSA in terms of the mecA gene type and reported mecA III as the most common type of the gene, while mecA IV was observed in the HA-MRSA samples. All the MRSA samples were sensitive to Vancomycin, Teicoplanin, Linezolid, Mupirocin and Folic Acid; however, a reduced sensitivity was observed among them to Rifampin, Co-trimoxazole, Clindamycin, Cephalexin, Tetracycline, Ciprofloxacin, Erythromycin and Gentamicin [25]. The first VRSA strain was reported in 2002 in the US and the first strain with a reduced Vancomycin-resistance was reported in 1997 in Japan, while the UK, France

and the US later reported the spread of VRSA strains [26, 27]. The various studies conducted to date in Iran have reported different rates of prevalence for Vancomycin-resistant Staphylococcus strains. In Iran, the resistance to Vancomycin was in the range 3% to 18.1% in many reports [28-29]. Comparison of the data obtained on the subject in Iran with the data obtained in other countries shows a low resistance to Vancomycin across the world, which makes the results of the present study on SA strains fully compatible with the global findings. Comparing the antibiotic resistance pattern obtained in this study with the patterns obtained in similar studies conducted on MRSA strains shows that the strains are quite resistant to antibiotics such as Penicillin, Amikacin, Gentamicin, Ciprofloxacin, Tobramycin, Erythromycin and Tetracycline and therefore do not provide effective treatment options, which could be due to their overuse in treating infections caused by different bacteria in the country. According to the results obtained of our study, 30.4% of MRSA isolates were resistant to Clindamycin, so this antibiotic is not suitable for treatment of this organism. None of the specimens collected in the present study were resistant to Vancomycin. A screening program for identifying MRSA types that were associated with a decreased sensitivity to Vancomycin was implemented in 1996 in Japan across 195 teaching hospitals. Of the 970 cases of MRSA, 13 [1.3%] showed a reduced resistance to Vancomycin [30]. Tiwary et al. [2006] reported six VISA strains in their study [31]. These findings are not consistent with the results of the present study. It can therefore be concluded that there is still no resistance to Vancomycin in Iran. All the strains examined in 2005 by Sancak in Turkey were sensitive to Vancomycin [32], which is consistent with the results of the present study.

According to a survey by mec/A gene method in Nigeria all isolated Staph A from nose of healthy college were sensitive to clindamycin that is in accordance of our study that30.4% of isolates were resistance to clindamycin ,so in Nigeria we can use clindamycin as first line antibiotic therapy but in our region not [33] .

Vidya Pai and co workers in India showed that in tertiary hospital [34] the prevalence rate of MRSA was 29.1% which is relatively in accordance of our study that 37% of our HASA were MRSA. Among their MRSA 18.8% were resist ant to clindamycin but as mentioned previously the clindamycin resistant rate in our study was 30% that higher than Indian study. Generally resistant to clindamycin is higher in our study than all above studies.

According to a survey in Taiwan based on the clinical and molecular definition [mec], 57.6% of Staphylococcus Aureus ocular infection was MRSA that is higher than our study [35]. In Taiwan study difference of antibiotic susceptibility pattern between CA-MRSA and HA-MRSA was statistically meaningful. In our study although

Antibiotic susceptibility pattern of CA-MRSA and HA-MRSA was different but not meaningful. Resistant to clindamycin was lower than our study. In a molecular epidemiology survey [mec/A] in Spain 38.7% of *Staphylococcus aureus* in a university hospital were MRSA that is in concordance with our study [36].

CONCLUSION

The increase in the prevalence of MRSA is caused by the overuse of antibiotics, which facilitates the colonization of MRSA. Moreover, the increase in the resistance to commonly-used antibiotics is a warning for a more reasonable prescription of sensitive antibiotics such as Vancomycin, so as to prevent the emergence of multidrug resistant MRSA.

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