Arch Pediatr Infect Dis.2013;**1**(1):4-8. DOI: 10.5812/pedinfect.5190

Archives of Pediatric Infectious Diseases
www.pedinfect.com
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Vancomycin and Linezolid Resistant Staphylococcus in Hospitalized Children

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ARTICLE INFO

Article type:
Original Article

Article history:
Received: 17 Apr 2012
Revised: 23 Jul 2012
Accepted: 11 Aug 2012

Keywords:
Methicillin-Resistant Staphylococcus Aureus
Linezolid
Vancomycin

ABSTRACT

Background: *Staphylococcus aureus* is a major cause of serious hospital and community acquired infections, particularly in colonized individuals.
Objectives: The study was carried out in a tertiary care center in Tehran, Iran to identify the frequency of hospital acquired methicillin resistant *Staphylococcus aureus* (HA-MRSA) colonization and its antibiotic susceptibility pattern and molecular characteristics.
Patients and Methods: This point-prevalence study was performed on 631 children who were admitted for at least 48 hours in different wards of Mofid children's hospital in Tehran, Iran**.** Samples from anterior nares of these children were taken with sterile swab and cultured. If *Staphylococcus aureus* (S. aureus) was isolated, methicillin resistance and antibiotic susceptibility pattern were diagnosed according to Center for Disease Control and Prevention (CDC) guidelines of 2011 and Clinical and Laboratory Standards Institute (CLSI), and molecular analysis were determined by minimum inhibitory concentration (MIC) and polymerase chain reaction (PCR) methods.
Results: Rate of colonization with *S. aureus* and methicillin resistant *Staphylococcus aureus* (MRSA) were 3.2% and 1.1% (1.1% of total and 35% of *S. aureus* isolates), respectively. All MRSA isolates were susceptible to rifampin and clindamycin. Resistance to vancomycin was reported in six *Staphylococcus* strains. Resistance to linezolid was detected in 19/20 *Staphylococcus*. Molecular analysis of isolates showed that all vancomycin resistant *S. aureus* isolates contained Van A or Van B gene, and 15/19 linezolid resistant strain was positive for chloramphenicol-florfenicol resistant gene (cfr gene).
Conclusions: The rate of MRSA colonization varies in any area**,** and the knowledge of acquisition risk factors and antibiotic susceptibility pattern are essential in prevention and treatment of MRSA infections. Based on our study, we suggest that clindamycin and rifampin are good choices in empiric treatment of patients suspected to have HA- MRSA infections until results of culture and antibiotic susceptibility pattern are prepared. In respect to the prevalence of linezolid resistance in this study, we suggest avoiding the use of linezolid as empiric therapy in HA-*Staphylococcus* infection.

▸ *Implication for health policy/practice/research/medical education:*
According to high expenses of these two antibiotics (vancomycin and linezolid) and significance of Staphylococcus antibiotic resistance as a gram positive microorganism in pediatric diseases, we decided to survey the sensitivity of organism to vancomycin and linezolid in children to improve our health policy and reduce high cost of treatment.

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DOI: 10.5812/pedinfect.5190

▸ *Please cite this paper as:*
Armin S, Rouhipour A, Fallah F, Rahbar M, Ebrahimi M. Vancomycin and Linezolid Resistant Staphylococcus in Hospilalized Children. *Arch Pediatr Infect Dis*. 2013; **1**(1): 4-8. DOI: 10.5812/pedinfect.5190

1. Background

Staphylococcus aureus is a major cause of serious hospital and community acquired infections, particularly in colonized individuals (1, 2). The first MRSA case was reported in the United Kingdom in 1961, shortly after methicillin was introduced to the clinical practice (3); now, the increasing rates of methicillin resistant *staphylococcus aureus* (MRSA) infection is a health problem (1, 2).

Nasal carriage of MRSA is a risk factor for developing hospital-acquired infections (4, 5). Huang and *et al.* reported MRSA colonization was associated with infection and has been shown that 29% of new carriers developed invasive infections in 18 months (6). Bacteremia due to *S. aureus* is three times more common in *S. aureus* nasal carriers than in non-carriers (7), and MRSA nasal carriage causes increased MRSA infections in ICUs (8, 9). Thus, knowledge of this colonization rate, their molecular characterization, and antibiotic susceptibility pattern in any area are useful data in prevention and treatment of serious MRSA infections (10).

Although MRSA infections have been reported frequently, population-based study about *S. aureus* and MRSA colonization rate are lacking, especially in Iran.

2. Objectives

This study was aimed to identify the frequency of *S. aureus* and MRSA colonization rate in hospitalized children in a tertiary care center (Mofid children hospital) in Tehran, Iran. Also we determined antibiotic susceptibility pattern, detection of vancomycin resistant *S.aureus* VRSA and linezolid resistant strains and molecular characteristics of the isolates.

3. Patients and Methods

A point-prevalence cross-sectional study was conducted during 4 months (April to July 2011). In this study, we surveyed 631 admitted children in all wards (except neonatal, NICU, and PICU wards) of Mofid children hospital in Tehran, Iran. HA-MRSA in The children were defined based on Huang *et al.* on criteria ,patient whose MRSA isolate is cultured more than 48 hr after admission; who has a positive history of previous hospitalization or surgery during 6 months ago; who has an underlying disease such as diabetes mellitus, chronic heart, liver, kidney, or lung disease; who is resident in a long-term health care facility within 6 months prior to the culture date; or who has an indwelling intravenous line, catheter, or any other percutaneous medical device at the time that the culture is taken (11). Patient's information such as age, gender, underlying disease, previous hospitalization and surgery, duration of hospitalization and use of antibiotics at the time of taking samples, history of oral or intravenous antibiotic usage during one month ago, and also the presence of intravenous device during sampling was recorded by the program-associated physician . Samples from anterior nares of these children were taken by sterile swab 48 hr after the admission and cultured initially in sheep blood agar. If gram positive cocci with positive hemolysis and positive catalase and coagulase were isolated, these were identified as *S. aureus* and were cultured in mannitol salt agar and DNase agar for proliferation. At this step, methicillin susceptibility of *S. aureus* isolates was confirmed by agar screen plate and MIC method. According to CDC guidelines of 2011, oxacillin and cefoxitin were used for methicillin susceptibility test. In addition to oxacillin and cefoxitin, susceptibility to rifampin, cotrimoxazole, cefazolin, clindamycin, vancomycin, and linezolid were performed for all *S. aureus* isolates with MIC method (antibiotic powders were provided by Sigma Co.). Purification of DNA was performed by i-genomic CTB DNA extraction Mini Kit iNtRON Biotechnology Inc., South Korea. Then, all purified DNA samples were checked by molecular analysis using PCR method by Accupower PCR Premix Bioneer, South Korea.

3.1. Statistical Analysis

Data analysis was performed using SPSS software. Statistical significance was assessed via the Pearson chi-square (or Fisher exact test) and t-test. *P*-value of less than 0.05 was considered to be statistically significant.

4. Results

Of 631 samples taken in this study, 20 cases (3.2%) were positive for *S .aureus* out of which 7 cases (1.1% of total and 35% of *S. aureus* isolates) were MRSA.

The highest colonization rate with *S. aureus* was demonstrated in infectious diseases ward (6 patients or 30%) out of which 2 cases were MRSA. The highest MRSA colonization rate was seen in infectious diseases and nephrology wards (2 cases or 28.5% of MRSA colonization rate). Distribution of *S. aureus* strains in different wards are shown in (*Table 1*).

As shown in *Table 2*, when non-colonized cases with *S. aureus* were compared with MRSA colonized patients, variables such as age, gender, underlying disease, previous hospitalization and surgery, duration of hospitalization and, the use of antibiotics at the time of taking samples were not statistically significant (*Table 2*).

History of oral antibiotic use during one month before sampling was significantly higher in non-colonized patients, (*P* =

Table 1. Distribution of Staphylococcus Strains in Wards

Ward	MSSA	MRSA
Infectious diseases	4	2
Nephrology	1	2
Surgery	2	1
GI	3	1
Pulmonary	1	1
Hematology	1	0
Neurology	1	0
Total	13	7

Abbreviations: GI, Gastrointestinal; MRSA, methicillin resistance *Staphylococcus aureus*; MSSA, methicillin sensitive *Staphylococcus aureus*

0.014) but correlation between MRSA colonization rate and this history was not statistically significant (*Table 2*).

In this study, all MRSA isolates were susceptible to rifampin and clindamycin and one case was resistant to vancomycin. The resistant strain was isolated from a 12-month-old boy who was admitted in the surgical ward for third stage repair of imperforated anus (pull-through surgery), had a positive history of previous surgery and hospitalization, and had received different courses of antibiotics. MRSA isolated from him was resistant to all antibiotics except to rifampin and clindamycin.

In this study, 6 MRSA isolates were resistant to linezolid, cefazolin, and co-trimoxazole (85.7%). In MSSA group, all were susceptible to rifampin, oxacillin, and cefoxitin. Five cases were resistant to vancomycin and all of them were resistant to linezolid (*Table 3*).

In molecular analysis of *S. aureus* isolates with PCR method, all 7 cases of MRSA had mec A gene. Of 19 resistant cases to linezolid (MSSA and MRSA), 15 cases carried cfr gene (13 strains of MSSA and 2 strains of MRSA). Of 6 resistant cases to vancomycin (MSSA and MRSA), 3 MSSA strains contained only Van A gene, 2 MSSA strains had Van B gene, and 1 MRSA strain contained both Van A and Van B genes (*Table 4*).

Table 2. Correlation Between *S. aureus* and MRSA Colonization Rate With Variables

	No. of Colonization (n = 611)	*S. Aureus* (n = 20)	*P* value	MRSA (n = 7)	*P* value
Median age (mo)	36	4	0.492	11.5	0.169
Gender, No. (%)			0.169		1
Female	321 (52.5)	12 (60)		4 (57)	
Male	290 (47.4)	8 (40)		3 (43)	
Duration of hospitalization, d	3.9 ± 0.15	3.4 ± 0.7	0.110	4.14 ± 2.19	0.190
Underling disease	79	1	0.495	1	1
History of previous hospitalization	229	7	0.972	3	0.674
History of previous surgery	187	3	0.972	2	0.674
Having catheter	552	17	0.435	7	1
History of oral antibiotic use	307	4	0.014	2	0.686
History of injectable antibiotic use	122	4	1	1	1
Antibiotic use during sampling	449	13	0.540	6	0.680

Abbreviations: MRSA, methicillin resistance *Staphylococcus aureus*

Table 3. Shows Antibiotic Susceptibility Pattern of All *S. aureus* Isolates.

Staph. Group	Antibiotic							
	Oxacillin	**Cefoxitin**	**Rifampin**	**Vancomycin**	**Cefazolin**	**Co-Trimoxazole**	**Linezolid**	**Clindamycin**
MRSA (n = 7)								
	Resistant	0	6	6	6	1	0	7
	Susceptible	7	1	1	1	6	7	0
MSSA (n = 13)								
	Resistant	3	13	7	2	5	0	0
	Susceptible	10	0	6	11	8	13	13

Abbreviations: MRSA, methicillin resistance *Staphylococcus aureus*; MSSA, methicillin sensitive *Staphylococcus aureus*

Table 4. PCR Results of *S. aureus* Isolates

Antibiotic	MSSA, No.	MRSA, No.	Results of PCR	MSSA, No.	MRSA, No.
Resistance to oxacillin and cefoxitin	0	7	All with mec A gene	0	7
Resistance to linezolid	13	6	15 cases with cfr gene	13	2
Resistance to vancomycin	5	1	3 cases with only Van A	3	0
			2 cases with only Van B gene	2	0
			1 case with both Van A and Van B genes	0	1

Abbreviations: MRSA, methicillin resistance *Staphylococcus aureus*; MSSA, methicillin sensitive *Staphylococcus aureus*

5. Discussion

Today, MRSA is recognized as a public health problem worldwide, being one of the main causative agents of hospital infections (10).

Various research papers have determined the prevalence and incidence of MRSA colonization rate in different areas of the world. In one study performed by Islam SI *et al.* in one eye care specialty hospital in Saudi Arabia (1999), the frequency of HA-MRSA colonization rate was reported as 0% (12). Armin Sh *et al.* reported the frequency of MRSA colonization rate in neonates as 5% in a children hospital in Iran (13).

In this study, the frequency of *S. aureus* and HA-MRSA colonization rate were 3.2% and 1.1%, respectively. The result of another study performed in 2007 in Imam Khomeini hospital in Tehran, Iran, of 356 *S. aureus* isolates, 149 (41.85%) strains were resistant to methicillin (14).

Thus, according to various studies performed in different areas of the world on different populations, the colonization rate of MRSA is variable in different settings. Colonization rate is important because nasal carriage of MRSA is a risk factor for developing hospital-acquired infections (4, 5).

In this study, all MRSA isolates were susceptible to rifampin and clindamycin, and resistance rate to cefazolin, co-trimoxazole, and linezolid was 85.7%. In another study performed by JB Sarma *et al.* in India, all MRSA isolates were resistant to erythromycin, trimethoprim, ciprofloxacin, gentamicin, and tobramycin; 85% were resistant to clindamycin and 96% to tetracycline, amikacin, and neomycin; all MRSA isolates were susceptible to teicoplanin and vancomycin (15). In another study in Pakistan, all the isolated MRSA organisms were uniformly susceptible to vancomycin, linezolid, and tigecycline. Other drugs which were found to be effective included chloramphenicol and rifampicin (16).

So, according to different studies performed worldwide, antibiotic susceptibility patterns of *S. aureus* and MRSA isolates vary in different areas; knowledge of this pattern in each area is essential for selection of the best choice for treatment of infections caused by these organisms.

In this study, one MRSA strain (14.2%) was resistant to vancomycin and contained Van A and Van B genes. In a recent report from Iran (14), 2 vancomycin-resistant strains in MRSA isolates were detected, one of which carried Van A and Van B. However, the other strain was resistant to vancomycin through other mechanisms such as vancomycin affinity trapping. We also found 5 vancomycin-resistant strains among MSSA samples, all of which carried Van A or Van B gene.

Among 20 strains of *S. aureus,* 19 cases were resistant to linezolid. Since linezolid is not used as a common antibiotic in our hospital, this result was unexpected but detection of chloramphenicol-florfenicol resistant (CFR) cases among 15/19 strain was a confirmatory finding as these strains are resistant not only to linezolid (LZD) but also to phenicols, lincosamides, pleuromutilins, and streptogramin A antibiotics and 16-membered ring macrolides (17, 18). Thus, selective focus on the use of any of these classes of drugs (such as clindamycin that is used frequently in our hospital) may lead to the spread of these resistant strains.

Several multicenter and multinational surveillance studies have shown that more than 99% of clinical strains of coagulase negative staphylococci and *S. aureus* still remain susceptible to linezolid (19), but in this study, all MSSA isolates were resistant to linezolid and possessed cfr gene. So, we propose that it could be due to transmission of this gene from veterinary isolates of *Staphylococcus warneri, Staphylococcus sciuri, Staphylococcus hyicus* (19), and *S. aureus,* or Entroccoci.

Although we didn't perform another molecular study for detection of other mechanisms that may be responsible for linezolid resistance in four CFR negative strains, because mutations in 23S rRNA remain the most commonly reported class of mutation leading to LZD resistance (20), we suggest that it is a possible mechanism for resistance in CFR negative strains. In a research in Colombia, where linezolid is not used routinely, the surveillance studies indicated that CFR resistance was still extremely rare in MRSA, although linezolid resistance in the absence of oxazolidinone exposure has been documented in *Enterococcus* spp. (21).

According to results of this study, we suggest that clindamycin and rifampin are good choices for empiric treatment of patients who acquire *S. aureus* or MRSA infections until the results of culture and antibiotic susceptibility pattern become available. However, because of high prevalence of tuberculosis infection in our country and rifampin being one of the most important drugs in anti-tuberculosis therapy, care should be exercised in using this drug for non-tuberculous infections, and to prevent occurrence of rifampin-resistant mycobacterium tuberculosis, physicians should list rifampin as the last choice in treatment of HA-MRSA infections. As we found linezolid resistance among MRSA and MSSA strains, we suggest antibiotic sensitivity test for all isolates before using this new and expensive antibiotic.

Acknowledgments

We thank all the staffs and associated workers from different wards and pediatric infections research center in Mofid children hospital for their cooperation.

Authors' Contribution

None declared.

Financial Disclosure

None declared.

Funding/Support

None declared.

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