Prevalence of methicillin resistant *Staphylococcus aureus* in school children of Pokhara

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ABSTRACT

Present study was carried out to find out the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in school children of Pokhara city in western, Nepal. A total of 184 randomly selected children younger than 15 years were included in the study. Nasal swabs collected were subjected to standard bacteriological culture. *S. aureus* isolates were identified by mannitol fermentation, coagulase positivity and DNase positivity. Antimicrobial susceptibility test was performed on muller-hinton agar (MHA) by modified Kirby-Bauer disc diffusion method. Out of total 184 nasal swabs, *S. aureus* was isolated in 31.0% (n=57). Among the isolates, 35.1% (n=20) were from male children whereas 64.9% (n=37) were from female. There was no significant sex difference in colonization of *S. aureus*. Out of 57 isolates, 56.1% (n=32) were MRSA. MRSA isolates indicated relatively high rate of resistance to antibiotic cloxacillin (68.7%) followed by ofloxacin (40.6%), tetracycline (15.6%), erythromycin (9.4%), ciprofloxacin (6.2%) and vancomycin (3.1%).This study showed a high prevalence of MRSA carriage in school children indicating the spread of MRSA in the community.

Keywords: *Staphylococcus aureus, MRSA, MHA, Modified Kirby-Bauer method.*

INTRODUCTION

Infections due to methicillin resistant *Staphylococcus aureus* (MRSA) are an increasing problem worldwide in community as well as hospital environment.[1,2] Community –acquired infections with *S. aureus* have, until very recently, been reliably treated with β-lactum antibiotics. This is in contrast to hospital-acquired infections, where for more than 20 years MRSA has been problem.[3] The extent of MRSA carriage in many communities is largely unknown and it varies in different geographical regions. Continuing surveillance is needed to assess the geographic distribution and epidemiology of infections therefore as to develop the strategies to control the spread of diseases.[2,3]

The resistance of *S. aureus* to methicillin is caused by the *mecA* gene which codes the low affinity 78-Kda penicillin-binding protein (PBP2a) or (PBP 2'). β-lactam antibiotic normally binds to PBPs in the cell wall, resulting in the disruption of synthesis of the peptidoglycan layer and death of bacterium. Since â-lactam cannot bind to low affinity PBP2', synthesis of the peptidoglycan layer and cell wall synthesis are able to continue.[4] MRSA infections often require systematic antibiotic therapy and are an important health care burden, since they increase treatment costs and patient morbidity. The spread of MRSA can also be potentially minimized by prevention of the risk factors such as previous antibiotic use, day care attendance, contact with a healthcare workers or nursing home resident, residence in a long-term care facility, diabetes mellitus, hospitalization, admission to an intensive care unit, intravenous drug use, invasive indwelling devices, hemodialysis or peritoneal dialysis, mechanical ventilation, endotracheal tube, tracheostomy tube, nasogastric tube, gastrostomy tube, or foley catheter, total parenteral nutrition or external feeding, surgical procedures, immunosuppression, chronic illness, and previous isolation of MRSA.[5] Vancomycin is the choice of drug for methicillin-resistant *S. aureus* isolates. Patients unable to tolerate vancomycin have been treated with fluoroquinolones, trimethoprim–sulfamethoxazole, clindamycin or minocycline.[6] Most of the work on *S. aureus* was focused mainly in Kathmandu Valley. Pant and Rai reported 43.8% *S. aureus* from nasal swabs of staffs in Nepal Medical College Teaching Hospital.[7] This study presents occurrence, distribution and prevalence of MRSA infections of children in Pokhara city.

MATERIALS AND METHODS

Sample collection: Nasal swabs of 184 children within age limit 1-15 years were collected for the purpose of the study during July to November 2007. The specimens were collected with the help of sterile cotton swabs available commercially. The swab was introduced 2-3 centimeter in the nasal cavity and rotated 4-5 times both clockwise and anticlockwise before withdrawal. Each sample was labeled with code number and various other

Table -1: Isolation of S. aureus in school children

Age group (yrs)	Male			Female		
	no. of nasal samples	no. of S. aureus isolates	% of S. aureus isolates	no. of nasal samples	no. of S. aureus isolates	% of S. aureus isolates
1-5	17	6	35.3	23	7	30.4
6-10	32	9	28.1	57	20	35.08
11-15	31	5	16.1	24	10	41.2
Total	80	20	25.0	104	37	35.6

information including age, sex, location, etc were also recorded. The sample was transported to the Laboratory of the School of Pharmaceutical and Biomedical Sciences within 1-2 hours for processing as have been described.[8,9]

Sample processing: Nasal swabs were inoculated onto mannitol salt agar (MSA) and incubated at 37°C for 24-48 hours for preliminary identification. *S. aureus* ferments mannitol and gives yellow distinct colonies. Mannitol fermenting colonies from MSA was subcultured on nutrient agar. Golden yellow colonies in the MSA indicate *S. aureus*, which is subsequently identified by gram's staining, catalase test, tube coagulase test, oxidative/fermentative test, methyl red / voges-proskauer test. DNase test was done for the further confirmation.[9,10]

Antibiotic susceptibility test: All the identified *S. aureus* isolates from nasal swabs were subjected to in-vitro susceptibility test by Modified Kirby-Bauer disc diffusion method.[11] The antibiotics used in the study were methicillin (5 mcg), tetracycline (30 µg), ciprofloxacin (30 µg), erythromycin (15 µg), ofloxacin (5 µg), vancomycin (30µg) and cloxacillin (30µg).

Quality control for the test: In the study, the accuracy of the over all testing procedure was monitored by using *S. aureus* ATCC 25923 as reference strain.

RESULTS

Among 184 students, 80 (43.5%) were male and 104 (56.5%) were female. Out of 184 nasal samples studied, *S. aureus* could be isolated from 57 samples (31.0 %). Among the isolates, 20 (35.1%) were from male whereas 37 (64.9%) were from female. There was no significant sex difference in colonization of *S. aureus*. The study showed that the highest colonization of *S. aureus* was found in the age group 6-10 (32.6%), followed by 1-5 (32.5%) and 11-15 (27.3%), respectively (Table -1).

Out of 57 isolates of *S. aureus*, 32 (56.1%) were methicillin-resistant. Among them 10 (31.3%) were from male and 22 (68.7%) were female children. There was no significant sex difference in MRSA distribution. The percentage of MRSA isolates in both male and female were found to be higher (100.0%) in age group of 1-5 years (Table- 2).

The rate of MRSA isolation was found to be 56.1% (32/57). MRSA isolates indicated a relatively high rate of resistance to antibiotics cloxacillin (68.8%) followed by ofloxacin (40.7%), tetracycline (15.6%), erythromycin (9.4%), ciprofloxacin (6.3%) and vancomycin (3.1 %) (Table -3).

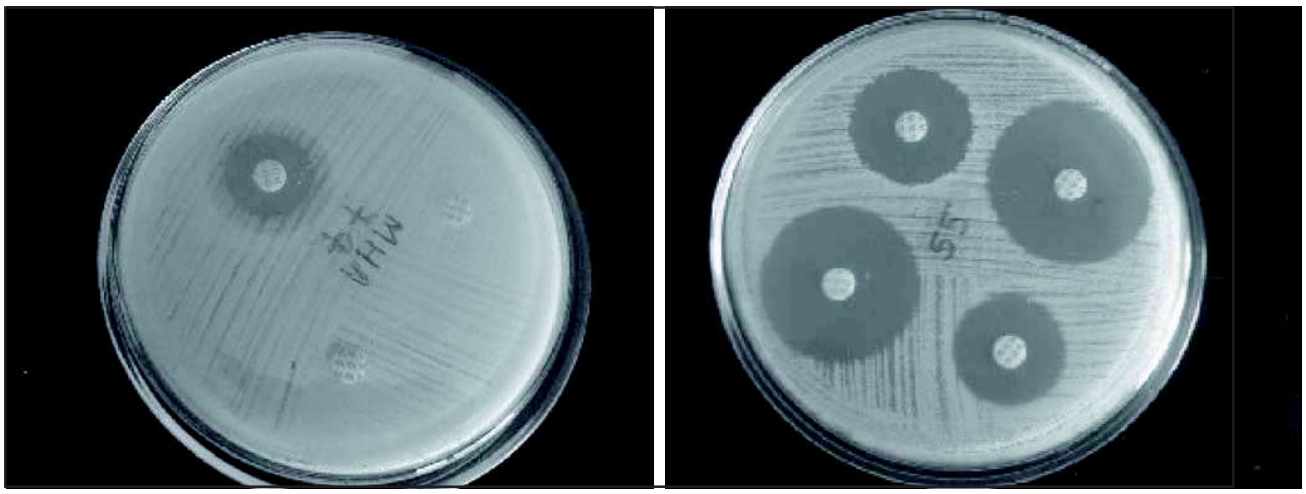

Fig. 1. Strain of *S. aureus* showing methicillin and vancomycin resistant (left) and sensitive strain of *S. aureus* with different antibiotics (right)

Table-2: Methicillin-resistant S. aureus in school children

Age Group (yrs)	Male			Female			Total		
	no. of S. aureus isolate	no. of MRSA isolate	% of MRSA isolate	no. of S. aureus isolate	no. of MRSA isolate	% of MRSA isolate	no. of S. aureus isolate	no. of MRSA isolate	% of MRSA isolate
1-5	6	6	100.0	7	7	100.0	13	13	100.0
6-10	9	4	44.4	20	14	70.0	29	18	62.06
11-15	5	0	0.0	10	1	10.0	15	1	6.7
Total	20	10	50.0	37	22	59.5	57	32	56.1

DISCUSSION

S. aureus remains a versatile and potent pathogen in humans, since it is one of the most common causes of nosocomial and community acquired infections.[12] Nasal carriage of *S. aureus* has been demonstrated to be a significant risk factor for nosocomial and community acquired infection in variety of population.[13, 14] *S. aureus* carriage has been demonstrated to be highly variable and age dependent and little is known of the factors that make one person to be a chronic carrier or a transient carrier. The highest rates have been found in newborns; the rates of *S. aureus* carriage tend to decrease with age.[14] Kaplan *et al*., demonstrated an increase in methicillin resistant in community acquired *S. aureus* infection in their 3 years prospective study.[15] In our study, one-third of the children are currently colonized by *S. aureus*. Another study conducted by Pant and Rai reported 43.8% *S. aureus* from nasal swabs of staffs in Nepal Medical College Teaching Hospital.[7] Yagci *et al*., reported 17.3% *S. aureus* in Turkish children.[16] Umera *et al*., found 36.0% *S. aureus* from nasal swabs of healthy volunteers.[17] Another study conducted in Taiwan 25.0% children had S. aureus isolated from nasal swabs.[18] So, the isolation of *S. aureus* from our study was more similar to other studies.

In our study the rate of MRSA isolation was found to be 56.1%. The MRSA carriage rate was found to be 100.0% in 1-5 years age group, 62.0% in 6-10 years age group and 6.7% in 11-15 years age group. Similar study in Taiwan showed 13.2% children are the carriage of MRSA.[18] Alfaro *et al*., found 22% of MRSA carriage in a group of children in South Texas.[19] In present study, MRSA isolates showed rate of resistance towards antibiotics cloxacillin (68.8%) followed by ofloxacin (40.7%), tetracycline (15.6%), erythromycin (9.4%), ciprofloxacin (6.3%) and vancomycin (3.1%). Pant and Rai reported rate of resistant of *S. aureus* towards antibiotics ampicillin (38.1%), erythromycin (33.3%), coxacillin (14.3%), gentamycin (9.5%) and methicillin (9.5%) respectively.[7] In comparison to other studies, the rate of isolation of MRSA and resistant rate towards different antibiotics from our study was slightly high. It may be due to certain limitations of our study. The first limitation of this study included the lack of knowledge of some risk factors associated with incidence and prevalence of MRSA infections in the community. Second, the samples selected for the isolation of *S. aureus* was randomly taken so there was no uniform distribution of children in different age groups. Third, due to limited time and resources, we did not take higher number of samples for study.

Table-3: Antibiotic Susceptibility pattern of MRSA isolates

Antibiotics	Total MRSA isolates	Sensitive		Resistant	
		n	%	n	%
Ciprofloxacin	32	30	93.8	2	6.2
Erythromycin		29	90.7	3	9.3
Tetracycline		27	84.4	5	15.6
Ofloxacin		19	59.4	13	40.6
Cloxacillin		10	31.3	22	68.7
Vancomycin		31	96.9	1	3.1

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