Original article

Methicillin Resistant Staphylococcus Aureus in patients visiting Western Regional Hospital, Pokhara

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Introduction: Methicillin Resistant *Staphylococcus aureus* (MRSA) continues to be a major cause of serious infection in hospitals and in the community worldwide. This study was performed to determine the prevalence of Methicillin resistant Staphylococcus aureus in healthy school children and patients visiting Western Regional Hospital of Pokhara.

Methods: The children from three Schools namely Janapirya Higher Secondary School, Sublime School and Ratnajyoti School and patients visiting Western Regional Hospital were selected for the study. This study was conducted by The School of Pharmaceutical and Biomedical Sciences during July to November 2007. One hundred eighty four Nasal swabs were collected from the healthy school children younger than 15 years old and one hundred clinical samples such as pus, wound swabs were collected from patients attending Western Regional Hospital. Collected nasal swabs and clinical swabs were analyzed for the growth of *Staphylococcus aureus* on mannitol salt agar (MSA). *S. aureus* was isolated and identified by mannitol fermentation, coagulase positivity and DNase positivity. Antimicrobial susceptibility test was performed on Muller-Hinton agar (MHA) medium by Modified Kirby-Bauer disc diffusion method.

Results: Out of 284 samples, 184 were nasal swabs, from healthy school children and 100 were clinical samples from patients attending Western Regional Hospital. Out of total nasal swabs (n=184), *S. aureus* was isolated from 30.97 % (n=57). Out of 57 isolates of *Staphylococcus aureus, isolated* from healthy school children 56.14 % (n=32) were MRSA. Out of 100 clinical samples isolated from hospital patients, S. aureus was isolated from 45% (n= 45).Out of 45 isolates of S. aureus, 75.55% (n=34) were MRSA. Prevalence of MRSA was statistically significant in clinical specimens in comparison with nasal swabs isolated from healthy school children ($P < 0.05$).

Conclusion: This study showed a high prevalence of MRSA carriage in school children and hospital patients of Pokhara valley and this may indicate the spread of MRSA in the community. Larger community based studies and regular surveillance of MRSA are needed to assist in the development of therapeutic guidelines for MRSA.

Key words: 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 *Staphylococcus aureus* have, until very recently, been reliably treated with â-lactum antibiotics. This is in contrast to hospital-acquired infections, where

for more than 20 years methicillin resistant *Staphylococcus aureus* (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 more accurately and to develop strategies that will improve the treatment and control the spread of diseases.

The resistance of *Staphylococcus aureus* to methicillin is caused by the *mecA* gene which codes the low affinity 78-Kda penicillin-binding protein (PBP2a) or (PBP 2'). ß-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 ß- 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. Several risk factors have been identified in patients who develop MRSA infection. They include 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 *Staphylococcus aureus* isolates. Patients unable to tolerate vancomycin have been treated with fluoroquinolones, trimethoprim–sulfamethoxazole, clindamycin or minocycline[6]. Little information is known about the prevalence of methicillin-resistant *Staphylococcus aureus* in context of Nepal. Most of the work on this bacterium was focused mainly in Kathmandu valley. Therefore this study was performed outside Kathmandu valley, at Kaski district. This study will provide information on the occurrence, distribution and prevalence of MRSA infections. The results of antibiotic susceptibility test would be helpful to suggest the most effective antibiotic to the clinicians to treat the infections caused by MRSA.

Materials and methods

This study was conducted in the Microbiology laboratory of The School of Pharmaceutical and Biomedical Sciences, Pokhara University, Pokhara during July to November 2007.

Materials: Mannitol salt agar (MSA), Amies transport media, Nutrient agar, DNase agar, MR-VP broth, Muller-Hinton agar was prepared by the instruction given on the media bottle.

Sample collection

Healthy school children

Nasal swabs of 184 healthy school children below 15 years old were collected for the purpose of the study. The specimens were collected with the help of sterile cotton swabs available commercially. The swab was introduced 2-3 inches 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 information including age, sex, location, etc were also recorded. The sample was transported in sterile condition within 1-2 hours for processing [7, 8].

Clinical sample

Clinical samples such as pus and wounds swabs were collected from 100 patients visiting Western Regional Hospital, Pokhara. The samples were collected with the help of sterile cotton swab. Each sample was labeled with unique code number and various other information including age, sex, location, antimicrobial therapy, etc were also recorded. Amies transport media with charcoal was used for the transportation of the clinical samples[7, 8].

Sample processing

Nasal swabs and clinical specimens were inoculated into mannitol salt agar (MSA) and incubated at 37 °C for 24-48 hours for preliminary identification. *Staphylococcus 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 *Staphylococcus aureus*, which is subsequently identified by Gram's staining, Catalase test, 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 *Staphylococcus aureus* isolates from different nasal swabs and clinical specimens 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 mcg), Ciprofloxacin (30 mcg), Erythromycin (15 mcg), Ofloxacin (5 mcg), Vancomycin (30 mcg) and Cloxacillin (30 mcg).

Quality control for the test

In the study, the accuracy of the over all testing procedure was monitored by using *Staphylococcus aureus* ATCC 25923 as reference strain.

Results

A total of 184 nasal swabs collected from the students (Under 15 years) of three different Schools of Pokhara metropolitan city and 100 clinical specimens (pus and wounds swabs) were analyzed in the Microbiology laboratory of The School of Pharmaceutical and Biomedical Sciences, Pokhara University. Different swabs collected were inoculated onto mannitol salt agar (MSA) and later sub cultured in nutrient agar. The bacterial isolates were further analyzed by various biochemical tests.

Out of 184 nasal swabs, processed for study, *Staphylococcus aureus* could be isolated from 57 samples (30.97%). Out of 57 isolates of *Staphylococcus aureus, isolated* from healthy school children 56.14 % (n=32) were MRSA. Out of 100 clinical samples isolated from hospital patients, S. aureus was isolated from 45% (n= 45).Out of 45 isolates of S. aureus isolated from hospital patients, 75.55 % (n=34) were MRSA. There was significant difference in MRSA prevalence between healthy school children and patients attending hospital. Overall percentage of isolation of MRSA isolates from both normal healthy individual and clinical samples was found to be 64.70%.

Table 1: Comparative study of isolation of MRSA from nasal swabs and clinical samples

Status	No. of MRSA isolates	No. of MSSA isolates	Total	Percentage of MRSA isolates
Healthy school children (nasal swabs)	32	25	57	56.14
Clinical Samples (hospital patients)	34	11	45	75.55
Total	**66**	**36**	**102**	**64.70**

MRSA= Methicillin Resistant *Staphylococcus aureus*

MSSA= Methicillin Sensitive *Staphylococcus aureus.*

The antibiotic susceptibility pattern of MRSA isolates from healthy school children varied slightly from hospital patients. The MRSA isolates from clinical samples (hospital patients) showed higher rate of resistance towards antibiotics like cloxacillin (85.29%), tetracycline (44.44%), erythromycin (38.23%), vancomycin (29.41%) and ciprofloxacin (23.52%) in comparison to MRSA isolates from healthy school children. There was slight difference in percentage of resistance toward ofloxacin between MRSA isolates from clinical samples (44.11%) and healthy school children (40.62%).

Table 2: Comparative study antibiotic susceptibility pattern of MRSA isolates from healthy school children and clinical samples (hospital patients)

Antibiotics	MRSA isolates from normal healthy individuals			MRSA isolates from clinical samples		
	Total MRSA isolates	No. of resistant	% of resistance	Total MRSA isolates	No. of resistant	% of resistance
Ciprofloxacin	32	2	6.25	34	8	23.52
Erythromycin		3	9.37		13	38.23
Tetracycline		5	15.62		15	44.11
Ofloxacin		13	40.62		15	44.11
Cloxacillin		22	68.75		15	85.29
Vancomycin		1	3.12		10	29.41

Discussion

Staphylococcus 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 *Staphylococcus aureus* has been demonstrated to be a significant risk factor for nosocomial and community acquired infection in variety of population[13, 14]. *Staphylococcus 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 *Staphylococcus aureus* carriage tend to decrease with age[13]. Kaplan *et al*. demonstrated an increase in methicillin resistant in community acquired *Staphylococcus aureus* infection in their 3 years prospective study[15]. In our study the rate of MRSA isolation was found to be 56.14% in healthy school children. This result suggests that community acquired MRSA is in escalating rate. Similar study in Taiwan showed 13.2% Children are the carriage of MRSA. [16] Alfaro *et al*., found 22% of MRSA carriage in a group of South Texas children [17]. The emergence of MRSA strains with multidrug resistant has posed challenges in the treatment of infection[18]. The SENTRY antimicrobial surveillance programme found that the prevalence of MRSA in hospital between 1997 and 1999 were very high in the countries like in Japan (71.6%), in Singapore (62.9%), Taiwan (61.1%) and Portugal (54.4%) [19]. In our study, we found that the prevalence rate of MRSA infection in patients visiting Western Regional Hospital was 75.55% (34) of 45 *Staphylococcus aureus* isolates. Study conducted in Tribhuvan university teaching hospital (TUTH) in 1993, Pokharel *et al*., reported the prevalence of 13% MRSA in 48.3% *Staphylococcus aureus* isolates[20]. MRSA was found in 31.43% of the total *Staphylococcus aureus* isolates from Kanti Children's Hospital and 11.76% from TUTH[21]. Rajbhandari *et al*. also reported 54.9% Methicillin-Resistant *Staphylococcus aureus* isolates at Bir Hospital[12]. MRSA was

found in 15.4% of the total *Staphylococcus aureus* isolates from Manipal Teaching Hospital[22]. Another study done in 2007 at Bir Hospital found that prevalence of MRSA was relatively high (75%) in burn ward[23].

In comparison to other studies, the rate of isolation of MRSA 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 *Staphylococcus 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 nasal swabs and clinical swabs (pus and wound swabs) for study.

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