Study of nosocomial isolates of *Staphylococcus aureus* with special reference to methicillin resistant *S. aureus* in a tertiary care hospital in Nepal

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ABSTRACT

To find out the prevalence of *Staphylococcus aureus* nosocomial infection and methicillin resistant *S. aureus* (MRSA), clinical samples from nosocomially infected patients were processed by following standard methodology in microbiology laboratory, Tribhuvan University Teaching Hospital, Kathmandu, Nepal. Of 149 *S. aureus* isolates, skin infection isolates contributed a major part 72.5% making nosocomial infection by *S. aureus* most prevalent in skin infection followed by lower respiratory tract infection 11.41% and urinary tract infection 8.7%. Overall MRSA prevalence was 45.0%. MRSA prevalence was 42.6% in skin infection, 82.3% in lower respiratory tract infection and 30.8% in urinary tract infection. MRSA infection was found associated with lower respiratory tract infection only. Highest occurrence of nosocomial infection was observed in female surgical ward, surgical out patient department, orthopedic ward, male surgical ward and maternity ward. MRSA isolation was high from lower respiratory tract of patients admitted in intensive care unit, coronary care unit, Sub-acute intensive care unit, intermediate coronary care unit, neurology ward and post-operative ward. Whereas methicillin sensitive *S. aureus* (MSSA) occurrence was higher in patients admitted in orthopedic, Surgical out patient department, and female surgical ward. The occurrence of MRSA did not differ with age but MRSA was found associated with male patients and MSSA was associated with female patients. Since MRSA prevalence was high, regular surveillance of MRSA and nosocomial infections should be done and universal precautions to control nosocomial infections should be followed.

Keywords: Nosocomial infection, methicillin resistant *S. aureus*, methicillin sensitive *S. aureus*, wards, gender.

INTRODUCTION

Early study reports report that 5.0% patients become infected during hospitalization and with increased use of invasive procedures; at least 8.0% of the patients now acquire nosocomial infections.[1] During invasive procedures pathogens that are present on medical personnel hands or in the instruments or that are acquired by the patient in the skin, respiratory tract, genitourinary tract, gets entry into the already weakened patients. These medical procedures bypass natural protective barrier against the entry of pathogens and provide an easy route for infection. Patients already colonized with hospital strains on admission are instantly put at a greater risk when they undergo such invasive procedure leading to nosocomial infections.

Nosocomial infections are caused primarily by opportunists, particularly by *Enterococcus* spp, *Escherichia coli*, *Pseudomonas* spp, *Staphylococcus aureus.*[2] These pathogens incorporated into the normal flora of healthcare workers are transmitted to patients during patient care and infections by such organisms are difficult to treat.

Methicillin resistant *S. aureus* (MRSA) is a major cause of morbidity and mortality around the world and has been a most common cause of nosocomial infection since late 1970s.[3] According to National Nosocomial Infection Surveillance in USA, the occurrence of MRSA causing nosocomial infection has increased from 2.0% in 1974 to 22.0% in 1995 and then to 63.0% in 2004.[4] In UK, 44.0% of *S. aureus* isolated from health care system are MRSA[5] and in Japan 60.0-70.0% of *S. aureus* are MRSA in inpatients.[6]

In USA and other countries, there has been a steady increase in the prevalence of MRSA strains.[7] Nosocomially acquired MRSA still remain low in some geographic locations, whereas in large cities the occurrence of nosocomial MRSA is much higher. In many large cities in USA, the infection caused by MRSA is 60.0% or higher.[8]

Data on the nosocomial infection by *S. aureus* in Nepal is not available. Hence present study was performed to find out the prevalence of nosocomial infection by *S. aureus* with special focus on MRSA.

MATERIALS AND METHODS

All samples submitted for culture and sensitivity from admitted patients in microbiology laboratory, Tribhuvan University Teaching Hospital, Kathmandu, Nepal during November 2007 to June 2008 were processed and inoculated onto chocolate agar (CA), blood agar (BA) and MacConkey agar (MA) as required by following standard methodology[9] and were identified. Gram positive cocci occurring in clusters and in short chains, catalase positive, oxidase negative, fermentative, Voges Proskauer positive, mannitol fermenter, clumping factor positive, DNase positive, coagulase positive and Staphytect plus latex agglutination (Oxoid, UK) positive were identified as *S. aureus*. History of all patients from whose clinical sample *S. aureus* had been isolated was taken and only those isolates that were isolated from nosocomial infection were identified as nosocomial isolates. Nosocomial infection was identified in those patients who developed infection after 48 hrs of admission in the hospital for a reason other than that for which the culture specimen was submitted, patient who developed infection after an invasive procedure, use of invasive and prosthetic devices, and those who developed infection 30 days after release from the hospital.[10]

The isolates (n=149) were processed for antibiotic sensitivity test by disc diffusion test by following Kirby Bauer method using antibiotic discs (Oxoid UK) recommended by FDA.[11] Isolates resistant to oxacillin (1 ìg) and cefoxitin (30 ìg) were identified as MRSA and those susceptible as methicillin sensitive *S. aureus* (MSSA).

Antibiotic discs used were: Penicillin (10 unit), oxacillin (1 ìg), cefoxitin (30 ìg), cotrimoxazole (1.25/23.75 ìg), rifamycin (5 ìg), gentamicin (10 ìg), ciprofloxacin (5 ìg), tetracycline (30 ìg), chloramphenicol (30 ìg), erythromycin (15 ìg), clindamycin (2 ìg) norfloxacin (10 ìg) and nitrofurantoin (300 ìg). In urine and urinary catheter isolates, erythromycin, clindamycin, chloramphenicol and ciprofloxacin were not used and novobiocin (5 ìg for identification), norfloxacin and nitrofurantoin were used.

The *S. aureus* isolates were grouped in 4 groups on the basis of infection sites. (Table-1).

Skin infection: pus, abscess, ear discharge, wound swab, bed sore Lower respiratory tract infection: sputum, endotracheal tube, tracheal aspirate Urinary tract infection: urine and urinary catheter. Others: blood, body fluid, high vaginal swab, tissue, ulcer. Data was analyzed by using statistical tool chi square test.

RESULT

Of 149 nosocomial *S. aureus* isolates, skin infection isolates contributed a major part 72.5% (n=108) making *S. aureus* nosocomial infection the most prevalent in skin infection. Further, isolates from pus comprised a greater part of the skin infection, that is, 90.7% (n=98/108). Lower respiratory tract infection was the second most prevalent site of nosocomial infection. It comprised 11.4% (n=17) of the total nosocomial infection. Sputum isolates made a large part of the lower respiratory tract infection, that is, 76.5% (n=13/17). Third in the order of prevalence of nosocomial infection was urinary tract infection comprising 8.7% (n=13) of urine and urinary catheter isolates. Other isolates from blood, body fluids, high vaginal swab, tissue (graft), ulcer grouped collectively comprised 7.3% (n=11) of the total nosocomial isolates (Table-1).

Of the total *S. aureus* nosocomial isolates, 45.0% (n=67) were MRSA and 55.0% (n=82) were MSSA. In skin infection, 42.6% (n=46/108) of the isolates were MRSA and MRSA was not found associated with skin infection (X^2=2.36, P>0.05). In the second most prevalent nosocomial infection site, the lower respiratory tract, MRSA comprised of 82.3% (n=14/17) of the isolates. All 4 isolates 2 each from endotracheal tube and tracheal aspirate were MRSA, whereas 76.9% (n=10/13) of the sputum isolates were MRSA. MRSA prevalence was associated with the nosocomial lower respiratory tract infection (X^2=7.10, P<0.05). MRSA was isolated from 30.76.0% (n=4/13) of nososcomially acquired urinary tract infection and in others group MRSA prevalence was 27.27% (n=3/11) (Table-2).

Highest occurrence of nosocomial infection by *S. aureus* was observed in female surgical ward (FSW 25), surgical out patient department (SOPD 22), orthopedic ward (ortho 21), male surgical ward (MSW 16) and maternity ward (mat 13).

All respiratory tract isolates (n=11) collectively from intensive care unit (ICU), coronary care unit (CCU), Sub-acute intensive care unit

Table-1: prevalence of MRSA and MSSA in different infection sites

Site of infection	MSSA	%	MRSA	%	Total	%
Skin infection	62 (56)	57.4	46 (42)	43.0	108	72.5
Lower respiratory tract	3 (3)	17.6	14 (10)	82.3	17	11.4
Urinary tract	9	69.2	4	30.8	13	8.7
Others	8	72.7	3	27.3	11	7.4
Total	**82**	**55.1**	**67**	**44.7**	**149**	**100.0**

Number in parenthesis in skin and lower respiratory tract infections are number of isolate from pus and sputum respectively.

Table-2: occurrence of MRSA and MSSA in different wards (LRT: Lower respiratory tract, UT: Urinary tract)

Ward	Skin		LRT		UT		Other		Total		Total
	R	S	R	S	R	S	R	S	R	S	
Orthopedic	7	13				1		1	7	14	21
ICU			6					1	6	1	7
CCU			1						1		1
SICU			1						1		1
ICCU		1	1						1	1	2
Neurology	1		1						2		2
Post operative	2		1						3		3
FSW	8	12				4		1	8	17	25
ANX I	4	2			1				5	2	7
Plastic and burn	2								2		2
ANX II	1	2			1			1	2	3	5
MMW	3		2	1		1	1		6	2	8
MSW	7	4	1	1	1	1	1		10	6	16
ENT	1	5							1	5	6
Surgical OPD	7	14		1					7	15	22
EYE	1	2							1	2	3
Paediatric	2	2							2	2	4
Maternity		4			1	2	1	5	2	11	13
Neonatal Unit		1								1	1
Total	**46**	**62**	**14**	**3**	**4**	**9**	**3**	**8**	**67**	**82**	**149**

(SICU), intermediate coronary care unit (ICCU), neurology ward (neuro) and post-operative ward (post op) were MRSA. The number of isolates was small in all wards except for ICU where the isolates number was 6. Isolates from skin infection from plastic surgery and burn ward (n=2) and from neuro ward (n=1) were MRSA.

In male medical ward (MMW), male surgical ward (MSW), Annex I, post op ward, the occurrence of MRSA was much greater than that of MSSA (24 MRSA versus 10 MSSA). On the contrary, most of the isolates from mat, ear nose throat (ENT) ward and neonatal unit (NNU) were MSSA (17 MSSA versus 3 MRSA). MSSA was also observed in greater occurrence in ortho, SOPD, and FSW (47 MSSA versus 22 MRSA) (Table-2).

Of 149 patients, 84 were male and 65 were female. Among male patients 53.6% (n=45/84) were infected with MRSA and obviously 46.4% (n=39/84) by MSSA. On the contrary, among female patients only 33.8% (n=22/65) were infected with MRSA and 66.2% (n=43/65) with MSSA. The infection by MSSA among male and female was almost equal (39 male versus 43 female) but the infection by MRSA among male was double to that among female (45/67 among male versus 22/67 among female) and was significant statistically (X^2=7.894, P<0.05). In other words, MSSA was more prevalent among female (X^2=6.7848, P<0.05), whereas among male patients the occurrence of MRSA and MSSA was almost equal. The occurrence of MRSA and MSSA did not differ with age (Table-3).

DISCUSSION

It has been stated that urinary tract is the most infected site of nosocomial infection followed by surgical wound, respiratory tract, skin especially burns, blood, gastrointestinal tract and then central nervous system.[2] In similar manner Center for Disease Control (CDC) 2007 has stated that urinary tract infection is the most prevalent nosocomial infection followed by surgical site infection and pneumonia.[12] In present study focused only on nosocomial infection by *S. aureus* skin infection most of which were related to surgery was the most prevalent nosocomial infection followed by respiratory tract and urinary tract infection.

Nosocomial MRSA prevalence obtained in present study was 45.0% which is much higher than that has been reported in the hospital isolates of *S. aureus* by different researchers in India 20.0-39.5%[13-16] and Nepal 15.4-29.0%.[17-19] Since present study is focused on the nosocomial isolates of *S. aureus*, the prevalence is expected to be greater than that among the hospital isolates of *S. aureus* reported in these reports.

Nosocomial infection by *S. aureus* was found associated with respiratory tract infection most of which were from the ICU. Of 7 isolates from ICU, 6 were from the lower respiratory tract and all were MRSA. In patients from

Table-3: occurrence of MRSA with age group and gender

Age group	Male patients			Female patients		
	MRSA	MSSA	Total	MRSA	MSSA	total
0-10		6	6	2	2	4
11-20	6	12	18	3	7	10
21-30	8	6	14	5	13	18
31-40	10	4	14	1	8	9
41-50	5	4	9	2	4	6
51-60	8	4	12	2	2	4
61 and above	8	3	11	7	7	14
Total	**45**	**39**	**84**	**22**	**43**	**65**

whom the MRSA were isolated, either iatragenic intubations (bypass of respiratory tract defense) were done or ventilators were used for patient care. It has been stated that in patients admitted in ICU, rate of acquisition of bacteria (present in ICU) increase with increase in the time they stay in ICU[20] supporting the present finding of the high occurrence of MRSA in ICU. Similar to 10.0% occurrence of MRSA in ICU of the total MRSA reported in tertiary care hospital in eastern Nepal,[17] MRSA isolate from ICU in present study accounted for 8.9% (n=6/67) of the total MRSA isolates. The prevalence of MRSA differed in each ward, however in certain wards where the prevalence was greater; almost all of the isolates were from the patients who had undergone surgery.

Significant infection by MRSA among male compared to female obtained in present study is concordant to another 1989-1997 report.[21] No significant association of nosocomial infection with gender and association of low birth weight neonate male with nosocomial infection with a ratio of 1.7:1 of male: female has been reported.[10]

The nosocomial infection places extra burden on individual patients and on health care system. Due to nosocomial infections, there is increased morbidity, including delayed wound healing, delayed rehabilitation, increased exposure to antimicrobial therapy and its potential adverse effect and prolonged hospitalization. The prolongation of hospital stay differs with the site of infection.[1] The nosocomial infections contribute to the emergence of resistant strains of organisms due to antibiotic selection pressure. The infected patients then act as a source of infection to other hospital patients and to healthcare workers.[1]

In totality, the cost of treatment of nosocomial infections is high. Studies have shown that the cost of treatment per infection with MRSA is more than double to that for MSSA.[22] Due to MRSA infection compared to MSSA in ICU, the length of hospital stay is almost double and mortality is more than double.[23] Therefore, due to the ever increasing MRSA infection in hospitals, related cost and ailments and its spread in other patients and as well as in the community via the health care workers, every hospital or health care settings should survey the MRSA prevalence and follow the universal precautions, the most important one being washing hand with alcohol based soap after each patient care.

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