Prevalence of Methicillin resistant *Staphylococcus aureus (MRSA)* among skin infection cases at a hospital in Chitwan, Nepal

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ABSTRACT

This study was carried out to see the methicillin resistance of *Staphylococcus aureus* isolated from patients with skin and soft tissue infection. In this study, a total of 600 *S. aureus* isolates isolated from 1,334 specimens from skin and soft tissue infection were included. For the screening of methicillin resistant of *S. aureus* (MRSA), specimens were inoculated on lipovitellin mannitol salt agar (LMSA) and MRSA were screened by testing resistance to oxacillin in AST plate following the standard bacteriological technique. The overall prevalence of MRSA was 68.0% (408/600). MRSA infection was higher among patients above 30 years of age. *Tibeto-Burmans* had high MRSA infection rate (72.5%) than *Indo-Aryans* (56.8%) and the rate was significantly higher among males (75.0%) than females (63.4%) ($p<0.05$). MRSA isolation rate was higher from wound (76.9%), followed by purulent exudates (67.7%) and abscesses (64.1%). The high prevalence of MRSA found in this study might be due to hospital based specimen.

Key words: MRSA, Soft tissue infections, LMSA, AST.

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) emerged in the 1960 as a cause of infection among patients exposed to the bacteria in health care centers.[1] It is a common cause of hospital and community-acquired infections worldwide. Reports of study on MRSA in Nepal, India and other countries show MRSA as an important cause of various infections.[2-12] Generally community- associated MRSA has primarily been described as a cause of skin and soft tissue infections, but it has also been associated with sepsis and tissue necrotizing pneumonia.[13] Therefore, prevention of staphylococcal infections assumes great importance. Carriage of *S. aureus* in nose appears to play a main role in epidemiology and pathogenesis of infections.[14,15] In general, presence of *S. aureus* infection rate among hospitalized patients is much higher (60.0-70.0%) as compared to that among community people (30.0-50.0%).[16-18] Treatment of *S. aureus* infections has now become more challenging with the emergence of MRSA, which are often multidrug resistant too. MRSA has been reported very frequently world- wide but emergence of *S. aureus* resistant to vancomycin also has raised now-a-days as a causative agent of community acquired infection.[19] Lipovitellin mannitol salt agar (LMSA) can be used to isolate and presumptively identify *S. aureus* from skin and soft tissue infected patients.[20] On LMSA, most strains of *S. aureus* produce a yellow (acid) zone due to fermentation of mannitol and produces opaque zone around the colony due to lipovitellin lipase activity and furthermore MRSA are detected in antibiotic sensitivity test plate by using 1.0% oxacillin discs in which *S. aureus* shows resistance to it.[21]

The reported prevalence of MRSA in Nepal shows an increasing trend; 29.1% in 1990[22] and 61.6% in 2003.[23] Report on MRSA as causative agent of skin and soft tissue infection is limitedly available from Nepal. So this study could be be beneficial for clinicians and health planning authority in Nepal.

MATERIALS AND METHODS

We have studied patients from Dermatology Department, CMS Teaching hospital, with purulent skin and soft tissue infections of less than 7 days duration, but all perirectal abscesses were excluded. Data were collected from January 01, 2008 to December 31, 2009 and all epidemiological information was obtained by interviewing the patient at the time of sample collection in the department of Microbiology with the active participation of dermatology Department. Total 1,336 samples were collected from single largest area of infection with purulent skin or soft tissue by using sterile cotton swab and processed within two hours of sample collection. Processing for culture and Antibiotic sensitivity test was done according to guidelines of National Committee for Clinical Laboratory Standards (NCCLS).[21]

Culture media was prepared by mixing LMSA provided by (HiMedia-MM118) with 20 mg of egg yolk/ml. Each

Table-1: MRSA positive rate among different sexes

Sex	Total	MRSA (n)	%	*p value*
Male	236	177	75	<0.05
Female	364	231	63.4	
Total	600	408	68	

90 mm Petri plate contained 18 to 20 ml of LMSA. The sample collected on sterile cotton swab was inoculated on LMSA and spread the material to isolate colonies by four flame technique. The LMSA plate was incubated at 37° for 24 hours and checked for colonies. If no growth, it was further incubated for 48 hours. All yellow colonies followed up with Gram's stain, catalase test and slide and tube coagulase test and serology were presumptively identified as *S. aureus*. MRSA screening was done while all *S. aureus* were isolated from clinical specimens using routine media like Blood agar, MacConkey agar during the corresponding period of time. For MRSA detection, Muller- Hinton agar incorporated with 4.0% NaCl and oxacillin discs (1.0% oxacillin) were used and incubated for 24-48 hours at 37°C. *S. aureus* showing resistance to oxacillin were reported as MRSA. The antibiotic sensitivity test of clinical isolates was done on Muller-Hinton agar using Kirby Bauer's disc diffusion method according to guidelines of NCCLS.[21] The isolates were tested against various antibiotics namely-penicillin-G (10µg), oxacillin (1µg), gentamicin (10µg), nitrofurantoin, erythromycin (15µg), ciprofloxacin (5µg), tetracycline (30µg), chloramphenicol (30µg), cotrimoxazole (25µg), amoxyclav (30µg) and vancomycin (30µg). These antibiotics were obtained from HiMedia India.

RESULTS

Among 1,336 skin and soft tissue samples collected for culture and sensitivity, 1,124 showed aerobic growth where as 212 did not show any growth. (Suspected as slow growing bacteria, anaerobic bacteria or may be due to immune system involvement).

Out of 600 *S. aureus* isolates, 408 (68.0%) were confirmed as MRSA (Fig.1). The MRSA infection rate was higher among patients of above 30 years age (88.1% among patients of 30-50 years age and 78.1% among >50 years age) compared to 57.3% among <30 years age (p<0.05) (Table-3). Result showed that MRSA infection rate was higher among males (75%) than females (63.4%) (p<0.05) (Table-1) and *Tibeto-Burmans* showed high MRSA infection rate (72.5%) than *Indo-Aryans* (56.8%) (p<0.05) (Table-2). Based on specimen, MRSA was highly isolated from infected wound (76.9%), followed by purulent exudates (67.7%) and then from abscesses (64.1%) (Table-4). Result of antibiotic sensitivity test showed the resistance pattern of *S. aureus* to different antibiotics (including erythromycin 75.3%, ciprofloxacin 70.0% and vancomycin 21.0%). This study indicates the emergence of vancomycin resistant *S.aureus (VRSA)* also (Table-5).

Table-2: MRSA positive rate among different Races

Race	Total	*MRSA* (n)	%	*p value*
Tibeto-Burmans	426	309	72.5	<0.05
Indo-Aryans	174	99	56.8	
Total	600	408	68	

DISCUSSION

MRSA has emerged as the most common identifiable cause of skin and soft –tissue infections in several areas of Nepal. As more than 80.0% patient with skin and soft tissue infections associated with MRSA in this study received empirical therapy, this finding suggests a need to reconsider empirical antibiotics choice for skin and soft tissue infections in areas where MRSA is prevalent.

The distribution of MRSA varies according to factors such as population, areas studied, the use of different culture techniques and different interpretation guidelines etc. Prevalence of MRSA among school children in Pokhara was found to be 56.1% [7] and other study on MRSA in TU Teaching Hospital revealed its prevalence to be 44.9% among health care workers.[24] Similarly other study on MRSA in Birgunj was found 57.1%.[5] The other study on MRSA among patients in Chitwan showed its prevalence to be 39.6%.[4] We found prevalence of MRSA among skin infection cases attending hospital to be 68.0% in Chitwan which is higher compared to other studies in Nepal. But study conducted at Bir hospital revealed prevalence of MRSA infection 55.0% among outpatients and that 76.0% within the hospitalized patients.[23] It is almost similar to our finding. While prevalence of MRSA was found 40.8% from the skin and soft tissue infected patients among community people which is less compared to it among hospital based cases.[25]

Our questionnaire was designed to establish demographic and associated factors related to *S. aureus*

Table-3: MRSA positive rate among different age groups

Age group	Total	*MRSA*(n)	%	*p value*
<30 yrs	382	219	57.3	<0.05
30-50yrs*	186	164	88.1	
>50 yrs*	32	25	78.1	*0.056
Total	600	408	68	

*(p>0.05)

Table-4: MRSA positive rate among different specimens

Sample	Total	*MRSA* (n)	%	*p value*
Infected wound	156	120	76.9	<0.05
Abscess*	354	227	64.1	
Purulent exudates*	90	61	67.7	*0.13
Total	600	408	68	*(p>0.05)

carriers and it's cause for infection of skin and soft tissues. High prevalence of MRSA found in our study may be due to hospital based specimens which might be due to long stay in the hospital, instrumentation and or invasive procedures which is similar to finding of other study in US.[18] *S. aureus* infection rate varies according to race, sex and age.[26] Our study showed that there is remarkable variations in MRSA infections among different races (*Tibeto-Burmans* had higher than *Aryans (p<0.05).* Race wise *Tibeto-Burmans* generally belong to low socio-economic status and are illiterate among Nepalese communities compared to *Indo-Aryans*, so lack of awareness and failure to complete full course of antibiotics treatment might have led to development of drug resistance and hence significant variation in MRSA isolation rate among these different ethnic groups.

Sex wise significantly high MRSA isolation from male cases compared to that from females (p<0.05) found in this study might be due to active involvement of males in outdoor activities including agricultural work. Due to this reason, males are more likely to get early community acquired infections than females and due to lack of knowledge, comparatively over use of antibiotics without prescriptions and its incomplete course among males might have led to high MRSA isolation from them compared to females.

Similarly age wise isolation of MRSA was found significantly high among people>30 years age compared to <30 years age(p<0.05) which is similar to findings from patients at Birgunj hospital.[5] Again empirical therapy or indiscriminate use of antibiotics in these communities might be responsible for drug resitance which developed slowly and might have appeared in later part of life while no significant difference in isolation of MRSA among people >30 years and >50 years age was found in this study(p=0.056), which might be due to less sample size.

Significant variation in MRSA isolation rate from infected wound and abscesses as well as that from infected wound and purulent exudates was obtained (p<0.05) which might be due to more chances of MRSA infection in deep seated lesions compared to superficially infected wounds.[27] while it was insignificant from cases of abscess and purulent exudates (p=0.13) again which might be due to less sample size included for purulent exudates cases. In addition, studies have shown that immunosuppression also may result to more chances of MRSA infection.[28] As patients included in this study were hospital-based, isolation rate of MRSA among skin and soft tissue infected cases is found to be high compared to findings of studies done in other places. [3,4 29-34,] Only skin infection cases included in this study might be other factor for this high prevalence of MRSA, which is almost similar compared to findings of other studies from different places.[23,3] Inoculation of *S. aureus* in LMSA is ideal, which produces yellow colonies due to mannitol fermentation after 24 hours of incubation and also larger, when compared with routinely used mannitol salt agar and makes easy to identify the large colonies with lipase activity.[20] It is said 100% sensitive and specific in differentiating coagulase negative Staphylococci from coagulase positive *S. aureus*.

MRSA isolation rate was high during August to December from different skin and soft tissue infected patients which is similar to findings of study in other places too.[24] In these months, due to favorable conditions *S. aureus* colonize very fast due to endogenous –autoinfection.[35] So all patients as well as health care workers should be instructed to maintain personal hygiene, the most important being washing hands with soap-water, and patients should be treated with antibiotics. This high prevalence

Table-5: Antibiotic susceptibility patterns of Methicillin sensitive and resistant *S. aureus*

S.No.	Antibiotics	Sensitive	Intermediate Sensitive	Resistant
		n. (%)	n. (%)	n. (%)
1.	Penicillin-G (10µ)	16 (2.7)	00 (00)	584 (97.3)
2.	Oxacillin (1µg)	192 (32.0)	00 (00)	408 (68.0)
3.	Gentamicin (10µg)	271 (45.2)	09 (1.5)	320 (53.3)
4.	Erythromycin (15µg)	148 (24.7)	00 (00)	452(75.3)
5.	Ciprofloxacin (5µg)	170 (28.3)	10 (1.7)	420(70.0)
6.	Tetracycline (30µg)	159 (26.5)	00 (1.5)	432 (72.0)
7.	Cotrimoxazole (25µg)	24 (4.0)	00 (00)	576 (96.0)
8.	Amoxyclav (30µg)	282 (47.0)	00(00)	318 (53.0)
9.	Vancomycin (30µg)	468 (78.0)	06 (1.0)	126 (21.0)
10.	Chloramphenicol (30µg)	50 (8.3)	00 (00)	550 (91.7)
11.	Nitrofurantoin(100 mcg)	269 (44.8)	10 (1.7)	321(53.0)

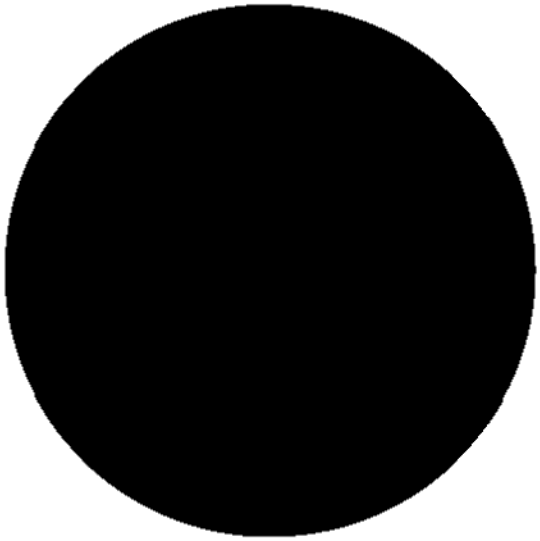

Fig. 1. Pie-chart showing prevalence of MRSA

of MRSA might be due to several contributing factors like- indiscriminate use of antibiotics, lack of awareness and unethical treatment before coming to the hospital. Report of this research showed B-lactam antibiotics like penicillin and cephalexin are ineffective drugs against *S. aureus*. Penicillin resistance was > 97.0% and quinolone - ciprofloxacin was also found to be resistant (70.0%). When we correlate with other's findings, ciprofloxacin resistance is steadily increasing (from 39.0% in 1992 to 68.0% in 1996).[35]All clinical isolates of this study have shown that only 78.0% of *S. aureus* were susceptible to vancomycin where as sensitivity to other drugs was also poor. This indicates that vancomycin resistance is also getting spread day by day, both in community as well as hospital settings. Therefore it requires continuous isolation and identification of *S. aureus* from carriers, patients and health care workers, so that regular monitoring and routine testing of other newer glycopeptides like teicoplanin should be carried out against it. However detection of VRSA by disc diffusion is not accurate method[36] which we have used in this study for MRSA detection. Similarly isolation of VRSA may be due to lab markers of severe/ chronic diseases (haemoglobin, haematocrit, leukocyte count, platelet count and serum albumin) among cases during sample collection.[37] So, lack of certain information in the collection of medical records might be our limitation of this study which was not kept in mind during research plan. Recent or past skin infection within 12 -months is significantly associated with risk of being MRSA carrier.[38] This might be most possible reason for high prevalence of MRSA among skin infected cases in this study as almost of the cases had history of prior skin infections.

Report of this study indicates necessity of regular surveillance of hospital associated infections including monitoring antibiotic sensitivity pattern of *S. aureus a*nd formulation of definite antibiotics policy to reduce the incidence of MRSA and multidrug resistant bacterial infections as well.

ACKNOWLEDGEMENTS

Authors are grateful to dermatology Unit, CMS Teaching Hospital for their cooperation during this study .We also thank Mr. P.B. Deshpande for his technical support.

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