Methicillin-Resistant *Staphylococcus Aureus*: Prevalence and Current Susceptibility Pattern in Sikkim

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ABSTRACT

Background: Prevalence of methicillin-resistant S*taphylococcus aureus* (MRSA) strains is reported to be increasing globally. **Objectives:** The study was conducted to find the magnitude and antibiotic susceptibility pattern of MRSA infection in a referral tertiary care teaching hospital of Sikkim, India. **Materials and Methods:** In this cross sectional study, 827 clinical specimens were collected from different departments of Central Referral Hospital. One hundred and ninety-six carrier screening nasal swabs were obtained from health care workers of the hospital. Subsequently, the antimicrobial susceptibility test was performed for the confirmed MRSA isolates as per Clinical and Laboratory Standards Institute (CLSI). **Results:** Methicillin resistance was seen in 152 isolates of *S. aureus* – 111 from clinical specimens and 41 from carrier screening samples. MRSA positivity among males was significantly higher than females. Extremely significant MRSA-positive cases were observed from ages less than 30 years, in-patient cases, particularly with a stay of more than 15 days and with a previous history of intake of broad spectrum antibiotics. Incidentally, there was no significant difference of MRSA positivity with a previous history of hospitalization. The extent of MRSA and drug resistance pattern was significantly different among various samples of *S. aureus*-positive isolates. The strains tested exhibited decreased susceptibility to vancomycin and imipenem. Most vulnerable of the carrier were the cleaners, that was a significant observation. Incidentally, there was no resistance in the carriers to both vancomycin and imipenem. **Conclusion:** MRSA is prevalent in our hospital and strains resistant to methicillin and vancomycin were quite high.

Key words: Antibiotic susceptibility, MRSA, Sikkim

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is the leading cause of gram positive bacterial infections and produces a wide spectrum of diseases, ranging from minor skin infections to fatal necrotizing pneumonia. Although *S. aureus* infections were historically treatable with common antibiotics, emergence of drug-resistant organisms is now a major concern. Methicillin-resistant *Staphylococcus aureus* (MRSA) was endemic in hospitals by the late 1960s, but it appeared rapidly and unexpectedly in communities in the 1990s and is now prevalent worldwide.[1,2]

S. aureus is notorious for its ability to become resistant to antibiotics. Infections that are caused by antibiotic-resistant strains often occur in epidemic waves that are initiated by one or a few successful clones. MRSA features prominently in these epidemics. Historically associated with hospitals and other health care settings, MRSA has now emerged as a widespread cause of community infections. Community or community-associated MRSA (CA-MRSA) can spread rapidly among healthy individuals. Outbreaks of CA-MRSA infections have been reported worldwide.[3]

The frequency of MRSA infections continues to grow in hospital-associated settings, and more recently, in community settings globally. The increase in the incidence of infections due to *S. aureus* is partially a consequence of advances in patient care and also of the pathogen's ability to adapt to a changing environment. Infection due to *S. aureus* imposes a high and increasing burden on health care resources. A growing concern is the emergence of MRSA infections in patients with no apparent risk factors.[4] The growing problem in the Indian scenario is that MRSA prevalence has increased from 12% in 1992 to 80.83% in 1999.[5] MRSA in tonsils may serve as a potential source for

Access this article online

Quick Response Code:

Website: www.jgid.org

DOI: 10.4103/0974-777X.77289

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the spread of these organisms to other body sites as well to other individuals.[6] MRSA is prevalent in many hospitals and often reflects the difficulties in hospitals and the health service generally, in terms of the control and prevention of healthcare-associated infection.[7] Multidrug-resistant bacteria, such as MRSA, are endemic in healthcare settings in the United States and many other countries of the world. Nosocomial transmission of MRSA serves as a source of hospital outbreaks, and recent reports of vancomycin resistant *S. aureus* strains in the United States emphasize the need for better control of MRSA and other resistant bacteria within healthcare settings.[8]

Although prevalence of MRSA strains is reported to be increasing, neither there was any history of MRSA in patients in our set up nor there was any study of MRSA prevalence reported from the Northeastern India. We had tried to demonstrate and compare the unique epidemiological features of the magnitude and antibiotic susceptibility pattern of MRSA infection in our patients. Additionally, we were looking for MRSA in health care providers to find the probability of risk among them.

MATERIALS AND METHODS

A cross sectional study was conducted in a referral tertiary care hospital of Sikkim, India during January to December 2006. Total of two hundred and ninety-one clinical specimens were subjected to MRSA screening using conventional microbiological methods. Subsequently, the antibiotic sensitivity test was performed for the confirmed MRSA isolates. A control group was selected from the health care providers working during that period of study to observe the extent of nosocomial spread. The study was done in close liaison with the Hospital infection control committee and managed as per the Revised Guidelines for MRSA Control. Institutional ethics committee approved the study. The main outcome measures were the positive cases of MRSA. All the patients or their caregivers were explained about the purpose of the study and were ensured strict confidentiality. Written informed consents were taken from each of the patients or their caregivers prior to the study. They were given the options of not to participate in the study if they wanted. The cases were referred to the Department of Microbiology, Central referral hospital, Gangtok from different in-patients and out-patients departments based on their clinical judgements for investigation and confirmatory diagnosis.

The standard microbiological methods were followed in this study during culture and antibiotic sensitivity test following universal precautions.

The clinical specimens and carrier samples were inoculated into 5% sheep blood agar (Hi Media, Mumbai, India)., Mac Conkey's agar (Hi Media, Mumbai, India). Mannitol salt agar was used as a selective medium for *S aureus*. These were incubated at 37°C for 24 hours. *S. aureus* was identified based on Gram's stain morphology, colony characteristics, and positive catalase and coagulase tests. The *Staphylococcus* species were identified by both slide and tube coagulase test. Slide coagulase was done for the detection of clumping factor present in *S. aureus*. Tube coagulase test modified from Gillespie (1943) was used in our study for the production of free coagulase enzyme using *S. aureus* NCTC 6571 as control strain.

All isolates were identified as *S. aureus* according to standard methods.[9] All the confirmed *S. aureus* strains were tested for methicillin resistance by the Kirby Bauer disk diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines. One microgram Oxacillin disk was used on Muller Hinton agar with 4% NaCl. Incubation temperature was 35°C for 24 hours. Zone diameter of the test strain was measured in millimeter with a scale. The isolates were considered methicillin resistant if the zone of inhibition was 10 mm or less.[10] Antibiogram was performed by the Kirby Bauer Disc Diffusion method as per CLSI Standards against the following antibiotics-penicillin G (10 units), ampicillin (10 mcg), erythromycin (15 mcg), tetracycline (30 mcg), gentamicin (10 mcg), netilmicin (30 mcg), vancomycin (30 mcg), ciprofloxacin (5 mcg), and imipenem (10 mcg). Information on MRSA was disseminated in health education sessions to complement the findings of study.

Statistical analysis

The data collected were thoroughly screened and entered into MS-Excel spread sheets and analysis was carried out. Procedures involved were transcription, preliminary data inspection, content analysis, and interpretation. Percentages were used in this study to analyze epidemiological variables and statistical significance tested.

RESULTS

Overall, a total of two hundred and ninety-one clinical specimens of *S. aureus* that were subjected to MRSA screening were collected from 156 males and 135 females. The MRSA positivity among males was significantly higher than the female counterparts [Chi square statistic (with Yates correction): 8.426, d.f.1, P=0.0037]. Extremely significant higher MRSA positive cases was observed from ages less than 30 years [Chi square statistic (with Yates

Table 1: Characteristics of patients with MRSA infection

Characteristics		Total cases *n* (%) (*n*=827)	*Staph. aureus* positive cases (%) (*n*=291)	MRSA positive cases (%) (*n*=111)	Chi square analysis
Sex	Male	539 (65.17)	156 (53.61)	72 (64.86)	8.426, d.f.1, *P*=0.0037
	Female	288 (34.83)	135 (46.39)	39 (35.14)	
Age	<30 yrs	629 (76.06)	168 (57.73)	94 (84.68)	51.651, d.f.1, *P*<0.0001
	>30 yrs	198 (23.94)	123 (42.27)	17 (15.32)	
Patient registered as	Inpatient	586 (70.86)	160 (61.86)	87 (78.38)	38.170, d.f.1, *P*<0.0001
	outpatient	241 (29.14)	131 (45.02)	24 (21.62)	
Duration of stay in hospital	>15 days	285 (34.46)	107 (66.88)	89 (83.18)	27.037 d.f.1, *P*<0.0001
	<15 days	532 (65.54)	53 (33.12)	22 (41.51)	
Previous history of hospitalization	Yes	139 (16.81)	47 (16.15)	28 (59.57)	2.391 d.f.1, *P*=0.1220
	No	688 (83.19)	113 (38.83)	83 (73.45)	
Previous history of intake of broad spectrum antibiotic	Yes	596 (72.07)	170 (58.42)	99 (58.24)	67.911 d.f.1, *P*<0.0001
	No	231 (27.93)	121 (41.58)	12 (9.92)	

MRSA: Methicillin-resistant *Stapbylococcus aureus*

correction): 51.651, d.f.1, *P*<0.0001], in-patients cases [Chi square statistic (with Yates correction): 38.170, d.f.1, *P*<0.0001], with a stay of more than 15 days [Chi square statistic (with Yates correction): 27.037, d.f.1, *P*<0.0001], and previous history of intake of broad spectrum of antibiotics [Chi square statistic (with Yates correction): 67.911, d.f.1, *P*<0.0001]. Incidentally, there was no significant difference of MRSA positivity with a previous history of hospitalization in our study. [Chi square statistic (with Yates correction): 2.391, d.f.1, *P*=0.1220] [Table 1].

Methicillin resistance was seen in 152 isolates of *S. aureus,* 111 MRSA were isolated from clinical specimens, and 41 from carrier screening samples. The prevalence of MRSA was significantly different among various clinical specimens. It was found that 61.90% of these were from throat swabs followed by sputum (56.52%), blood (50%), urine (45.83%), and pus (27.05%) [Table 2].

The antibiotic sensitivity pattern of MRSA isolated from clinical specimens and carrier screening samples were found to be highly variable. Among 111 (38.14%) MRSA screened from clinical specimens, 7.22% were sensitive to penicillin, 4.81% to ampicillin, 4.12% to erythromycin, 35.40% to imipenem, and 20.27% to vancomycin. The resistance pattern was very high for vancomycin which was an alarming finding. However, from 41 (20.92%) MRSA screened from carriers all were sensitive to vancomycin and imipenem [Table 3].

Out of one hundred and ninety-six carrier screening nasal samples taken from different categories of health care staffs taken, it was found that forty-one (20.92%) were found to be methicillin resistant. Most vulnerable of them were the cleaners (51.35%). This was a significant observation (*P*=0.029). Methicillin resistance in attendants and nurses both were little above 25 percent. But incidentally in the antibiogram all of the isolates were sensitive to both imipenem and vancomycin. This was also an important observation [Table 4].

Table 2: Patterns of MRSA isolations

Source	No. of samples	Number *S. aureus* isolated (%)	Number MRSA isolated	Percentage of MRSA isolated
Pus	648	207 (31.88)	56	27.05
Sputum	38	23 (60.87)	13	56.52
Urine	36	24 (66.67)	11	45.83
Blood	64	16 (25.00)	8	50.00
Throat	41	21(52.38)	13	61.90
Total	827	291	111	38.14

MRSA: Methicillin-resistant *Stapbylococcus aureus*

DISCUSSIONS

In this study, we isolated 111 (38.14%) MRSA from 291 *S. aureus* from 827 clinical specimens and 41 (20.92%) MRSA were isolated from 196 carrier screening samples. As high as 61.90% of *S. aureus*-positive samples from throat swabs, 56.52% from sputum, 50% from blood, 45.83% from urine, and 27.05% from pus were MRSA. Another significant observation was high resistance to vancomycin (20.27% sensitive) and imipenem (35.40% sensitive). However, no resistance to vancomycin or imipenem was observed from carriers.

Brook *et al*, (2006) demonstrated that MRSA was isolated from 16% of the recurrently infected tonsils; seven isolates were recovered from the cores and two were isolated from the surface.[6]

In a study at Aligarh, India, it was shown that 35.1% of *S. aureus* and 22.5% of coagulates-negative staphylococcal isolates were resistant to methicillin. Highest percentage of MRSA (35.5%) was found in pus specimens (*n*=151). In case of both methicillin-resistant as well as methicillin-sensitive *Staphylococcal* isolates, zero resistance was found to vancomycin.[11] But, methicillin resistance is uncommon in

Table 3: Antimicrobial susceptibility patterns of MRSA

Antimicrobial Percent sensitive	Pus *n* (%) (*n*=207)	Sputum *n* (%) (*n*=23)	Urine *n* (%) (*n*=24)	Blood *n* (%) (*n*=16)	Throat *n* (%) (*n*=21)	Sensitivity among cases *n* (%) (*n*=291)	Sensitivity among carriers *n* (%) (*n*=41)
ampicillin	7 (3.38)	4 (17.39)	1 (4.17)	2 (12.50)	0	14 (4.81)	20 (48.78)
penicillin G	8 (3.86)	5 (21.74)	3 (12.50)	3 (18.75)	2 (9.52)	21 (7.22)	27 (65.85)
erythromycin	5 (2.42)	3 (13.04)	2 (8.34)	1 (6.25)	1 (4.76)	12 (4.12)	15 (36.59)
ciprofloxacin	9 (4.35)	7 (30.43)	5 (20.83)	1 (6.25)	1 (4.76)	23 (7.90)	10 (24.39)
netilmicin	6(2.90)	8 (34.78)	7 (29.17)	4 (25.00)	2 (9.52)	27 (9.28)	30 (73.17)
tetracycline	4 (1.93)	3 (13.04)	3 (12.50)	3 (18.75)	3 (14.29)	16 (5.50)	12 (29.27)
imipenem	57 (27.54)	17 (73.91)	13 (54.17)	8 (50.00)	8 (38.10)	103 (35.40)	41 (100)
Vancomycin	27 (13.04)	15 (65.22)	9 (37.50)	3 (18.75)	5 (23.81)	59 (20.27)	41 (100)

Table 4: Distribution of MRSA from carrier screening samples

Category	No. of samples	MRSA positive	Percentage of MRSA carriers
Doctors	32	1	3.12
Nurses	43	11	25.58
Technicians	37	8	21.62
Attendants	47	12	25.53
Cleaners	37	19	51.35
Total	196	41	20.92

community-acquired primary pyodermas in Mumbai.[12] In major southern districts of Tamilnadu, out of 906 strains of *S. aureus* isolated from clinical samples, 250 (31.1%) were found to be methicillin resistant. However, all strains of clinical and carrier subjects were sensitive to vancomycin.[13] A study from Mumbai, reports that the incidence of MRSA was 15.87%. All the MRSA strains isolated, however, were found to be sensitive to vancomycin.[14] Researchers in other part of the globe also observed that many of these MRSA isolates were becoming multidrug resistant and were susceptible only to glycopeptide antibiotics such as vancomycin. Low level resistance even to vancomycin was emerging.[15]

In a prospective surveillance study on children in the UK and Republic of Ireland, noted that the overall incidence rate of MRSA isolated from cases was 1.1 per 100,000 child population per year, 61% of the children were aged <1 year (a rate of 9.7 cases per 100,000 population per year).[16] Studies from England based on clinical data collected on 385 children first identified as having MRSA between January 1998 and December 2003. The researchers observed that there were 267 inpatients and 118 outpatients. The number of new cases of MRSA declined from 72 in 1998 to 52 in 2003, whereas hospital admissions increased. Ninety nine (37.1%) inpatients acquired MRSA outside the hospital; a further 90 occurred among 31 clusters of cases.[17]

A study from Taiwan delineated the clinical features of MRSA bacteraemia in infants hospitalized at the neonatal intensive care unit. Episodes of MRSA bacteraemia 1997 to 1999 were reviewed. The overall rate of MRSA bacteraemia was 1.05 per 1000 patient days during the 3 year period.[18]

Strength of the study

The study findings were shared with the hospital authorities to help them formulate a new antibiotic policy so that a uniform protocol is maintained as the modern concept is that primary caretakers have to use antibiotics as little as possible and as short as possible. This study was an eye opener for the hospital infection committee.

Limitations of the study

The limitation of our study was that we presented an analysis of MRSA prevalence in a tertiary healthcare center in Northeastern India. It would have been better if we could do a population-based study. The percentages were apparent only among all the patients within this referral center during the study period. The high rate of vancomycin should have been substantiated by MIC testing but we could not go for it because of lack of infrastructure and facilities in these parts of India.

Future direction of the study

We hope to undertake the study in community perspective to be acquainted with the actual prevalence and pattern of MRSA colonization and ascertain the extent of the menace at a larger level so that the information can be disseminated down the path, even to primary care health workers right at the grass root level before it becomes a public health problem.

CONCLUSION

To sum up, MRSA is prevalent in our hospital and is common in community-acquired infection in Northeastern India. There is a progressive increase in MRSA positivity and multi-drug resistance in strains of Staphylococci.

Theoretically, vancomycin is still the drug of choice for MRSA infections. But our study shows alarmingly high resistance of these strains to vancomycin also. The major reservoir of methicillin resistant staphylococci in hospitals is colonized/infected inpatients and colonized hospital workers. In our study, although vancomycin resistance was alarming but on the other hand all the carriers were sensitive to vancomycin. The field practitioners should be judicious enough in terms of use of antimicrobials so that the growing problem of antibiotic resistance of organism isolated does not reach a level of public health concern in this part of India. We recommend that frequent monitoring of susceptibility patterns of MRSA and the formulation of a definite antibiotic policy may be helpful in decreasing the incidence of MRSA infection. The dissemination of this information will help the treating clinicians for the primary care level physicians.

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How to cite this article: Tsering DC, Pal R, Kar S. Methicillin-resistant *Staphylococcus Aureus*: Prevalence and current susceptibility pattern in Sikkim. J Global Infect Dis 2011;3:9-13.

Source of Support: Nil. **Conflict of Interest:** None declared.