

Prevalence and Antimicrobial Susceptibility of Methicillin Resistant Staphylococcus in a Tertiary Care Hospital in Koshi Region (Northern Bihar)

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ABSTRACT

Introduction: Nosocomial infection is a major problem globally. Methicillin-resistant *Staphylococcus aureus* (MRSA) remains one of the most important causes of nosocomial infections worldwide. MRSA are the important agents causing nosocomial infections. The study was conducted in the Department of Microbiology to determine the prevalence of MRSA and antibiotic susceptibility pattern.

Material and Methods: This was a retrospective study conducted from July 2016 to August 2017 in a tertiary care hospital in Northern Bihar India. All isolates were identified in patients and data provided by the Clinical and Laboratory Standards Institute (CLSI) guidelines and antibiotic susceptibility pattern considered by Kirby Bauer disc diffusion method. The information was definitely recorded and analyzed using Microsoft Excel 2007 edition.

Results: 200 Staphylococcus strains isolate of were isolated from various clinical samples, Out of 200 *S. aureus* isolates, 73 (36.5%) were methicillin resistant *S. aureus* (MRSA) and 127 (63.5%) were methicillin-sensitive *S. aureus* (MSSA) in our labs. Although, the majority of the MRSA isolates were resulted from pus samples 30, however, the *S. aureus* isolates resulted from post-operative wound infection was mostly MRSA.

Conclusion: In conclusion, the importance of Isolation of MRSA patients and carriers in the hospitals, regular surveillance of hospital associated infections including monitoring antibiotic sensitivity pattern and strict drug policy for antibiotics may be helpful for reducing the incidence of these infections.

Keywords: MRSA, MSSA, Nosocomial Infection, Susceptibility Pattern.

MRSA in the developed countries has increased from 2% to 30%.⁵ Moreover, half of *S. aureus* in different states of India are methicillin resistant (multidrug resistant) posing major therapeutic challenge.⁶

MRSA now till date endemic in India. The incidence of MRSA varies according to the different region, 25% to 50%.^{7,8} MRSA is now one of serious concern for current therapeutic options for MRSA are few expensive, limited drugs now available like vancomycin, linezolid, teicoplanin, daptomycin and streptogramins. Another recently alarming sign has been that emergence of resistance to Vancomycin, although at a low level has been reported.⁹ Glycopeptides and linezolid continue to remain the mainstay of treatment for MRSA.

Both endemic and epidemic MRSA infections happen globally as infected and colonized patients in hospitals mediate the dissemination of these isolates and hospital staffs assist further transmission.¹⁰ Although many studies have been done in different regions on the prevalence and antibiogram of staphylococcus, but many of these studies have concentrated only on methicillin resistant staphylococcus. Our study has been carried to conduct to determine the prevalence of MRSA and antibiotic susceptibility staphylococcus aureus in the Koshi region (Northern Bihar).

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INTRODUCTION

Staphylococcus aureus is considered to be one of the most clinically important *staphylococcal* pathogens affecting humans, has acquired resistance to different types of antibiotics and is a leading cause of hospital and community acquired infections, manifesting from minor skin diseases to life threatening infections.^{1,2} Methicillin resistant *Staphylococcus aureus* was first time described in 1961, reported after one year of introduction of methicillin and has now emerged as one of the most important nosocomial pathogens especially in the last two decades.³

Now MRSA has emerged as one of the most important nosocomial pathogens.⁴ The percentage of hospitals isolating

MATERIAL AND METHODS

This study was done in July 2016 to August 2017 and based on retrospective data of samples sent from different wards and OPDs of Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar and Associated Hospital. Total strains of 200 *S. aureus* were isolated from pus, urine, sputum, wound swab, aural swab, blood, throat swab and urethral swab during July 2016 to August 2017. *Staphylococcus aureus* strains isolated from culture specimens from patients who have been hospitalized for > 48 hours were included in our study.

Staphylococcus aureus were characterised by their morphology on Gram staining, growth characteristics and coagulase production.

Clinical samples and bacterial isolates:-

During of the one year period 200 clinical isolates of *S. aureus* were collected from Our Microbiology Department, Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar and Associated Hospital. The bacteria isolated from patients in various wards and different specimens such as pus, urine, sputum, wound swab, plural swab, blood, throat swab and urethral swab and so on, were transported to Our Department were confirmed by standard microbiology tests including: Gram staining, catalase, slide and tube coagulase, mannitol fermentation and production of DNase enzyme. [Fig 1, 2, 3, 4, and 5]

Source of isolates

The source of the isolates were exudative specimens (pus, wound swabs, ear swabs and body fluids), blood, respiratory

Types of Sample	Samples Number	Samples Percentage
Pus	114	57.00%
Blood	51	25.70%
Urine	10	05.20%
Sputum	10	04.80%
Miscellaneous*	15	07.30%
Total	200	100

[Note*:- Miscellaneous samples include ear discharge, throat swab, conjunctival swab and wound discharges etc.]

Table-1: Different Sample-wise distribution of *S. aureus* isolates [n=200]

secretions and urine obtained from cultures of specimens from patients who had been hospitalised for > 48 hours.

Sample evaluation

A total of 200 consecutive, clinically significant, nonrepetitive *Staphylococcus aureus* strains were included in the study.

MATERIAL AND METHODS

The *Staphylococcus aureus* isolates in tertiary care hospital in Koshi region were subjected to susceptibility testing by disc diffusion technique according to the Clinical Laboratory Standards International (CLSI) guidelines with quality controls.¹¹ The antimicrobials tested in Central Pathology labs in tertiary care hospital were chloramphenicol (30µg), tetracycline (30µg), gentamicin (10µg), erythromycin (15µg), cotrimoxazole (25µg), cephalexin (30µg), ciprofloxacin (5µg), amikacin (30µg), cefotaxime (30µg) and vancomycin (10µg).

Screening for MRSA

Methicillin resistance was isolates by disc diffusion method using 30µg cefoxitin disk (Becton Dickinson). The diameter of the zone was, according to CLSI guidelines. Our isolate was considered to be an MRSA strain if cefoxitin inhibition zone diameter was < 21 mm.¹¹ Since *Staphylococcus aureus* can be a colonizer^{12,13}, special emphasis on the clinical significance of all the isolates. This was done by correlating with a Gram stained smear examination with the clinical history.

STATISTICAL ANALYSIS

The clinically data was analyzed using SPSS Data Editor Software, Chicago, version 20. The statistical methicillin resistant *S. aureus* isolates were evaluated using Chi-square test and p < 0.05 was considered as statistically significant.

RESULT

Among 200 *S. aureus* isolates included in our study, 114 (57%) were isolated from pus samples, 51 (25.70%) were isolated from blood, 10 (5.2%) were isolated from Urine, 10 (4.8%) were isolated from sputum, and 15(7.3%) were isolated from miscellaneous samples as shown in [Table 1]. Out of 200 *S. aureus* isolates, our finding 73 (36.6%) were methicillin resistant (MRSA) and 127 (63.4%) were

Sample	Staphylococcus aureus			Chi-Square (χ ²) & *p value $\chi^2 = \sum_{i=1}^r \sum_{j=1}^c \frac{(O_{ij} - E_{ij})^2}{E_{ij}}$
	Resistant to cefoxitin (MRSA) N = 73 (36.5%)	Susceptible to cefoxitin (MSSA) N = 127 (63.5%)	Total isolates N = 200 (100%)	
Pus due to any other cause, N(%)	30 (34.23%)	59(65.77%)	89(44.4%)	Chi ² =9.6316 DF=4; P=0.047113 statistically significant
Post operative Wound infection, N (%)	13(52.38%)	12 (47.62%)	25 (12.6%)	
Blood; and SNCU blood culture, N (%)	16 (31.78%)	35(68.22%)	51 (25.7%)	
Miscellaneous* Sample, N (%)	10(41.67%)	14(58.33%)	24 (12.1%)	
Urine due to any other cause, N (%)	04(36.36%)	07(63.63%)	11(05.2%)	

N = Number of isolates; MSSA = Methicillin sensitive *Staphylococcus aureus*; MRSA = Methicillin resistant *Staphylococcus aureus*; *p value < 0.05 was considered as statistically significant.

Table-2: Miscellaneous samples include ear discharge, throat swab, abdominal drain fluid, conjunctival swab and wound discharges etc.

Antibiotic tested	Total No. N = 200 (100%)	
	MARSA NO. (%)	MSSA NO. (%)
Vancomycin	0(00.00%)	200(100%)
Linezolid	0(00.00%)	200(100%)
Ciprofloxacin	158(79.05%)	42(20.95%)
Cefoxitin	50(24.76%)	150(75.24%)
Gentamicin	46(22.86%)	142(71.14%)
Cefuroxime	46(22.86%)	142(71.14%)
Amoxyclav	72(36.19%)	128(63.81%)
Amoxicillin	1(33.33%)	2(67.77%)
Doxycycline	152 (76.19%)	48(23.81%)
Levofloxacin	80(40%)	120(60%)

N = Number of isolates

Table-3: Resistance to individual antimicrobials in MRSA and MSSA isolated in Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar and Associated Hospital.

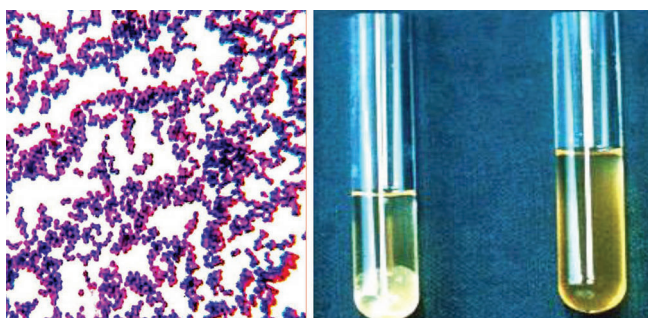


Figure-1: Staphylococcus aureus; Figure-2: tube coagulase test

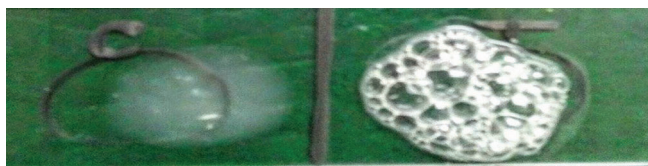


Figure-3: Catalase test



Figure-4: Slide coagulase test

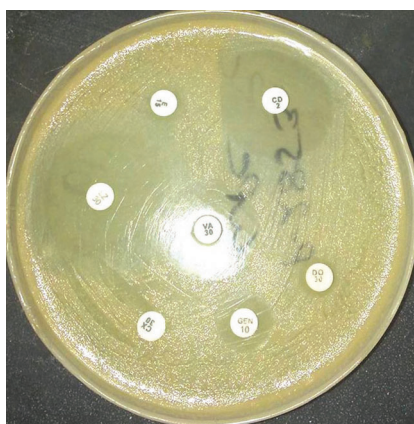


Figure-5: Antimicrobial susceptibility test

methicillin-sensitive *S. aureus* (MSSA). while, the majority of the MRSA isolates were derived from pus samples 30,

though, the *S. aureus* isolates derived from post-operative wound infection was mostly MRSA 13. This finding was found to be statistically significant ($P=0.008903$). [Table-2] Out of 200 *S. aureus* isolates of the MRSA 73 (36.5%) were derived from respectively Pus samples 30 (41.09%), the *S. aureus* isolates derived from Wound Samples were MRSA 13(17.80%), the *S. aureus* isolates derived from Blood Samples were MRSA 16 (21.91%), the *S. aureus* isolates resulted from Miscellaneous Samples were MRSA 10 (13.69%) and the *S. aureus* isolates resulted from Urine Samples were MRSA 4(5.47%). This finding was found to be statistically significant ($p=0.347491$).[Table-2]

The antimicrobial susceptibility tested all 200 *S. aureus* isolates. Among MRSA, resistance 100% sensitive to vancomycin and linezolid, but moderate sensitivity (71.14%) to gentamicin, cefuroxime and least sensitivity to (23.81%) doxycycline, (20.95%) ciprofloxacin as shown in [Table 3].

DISCUSSION

In emergence of multidrug-resistant *S. aureus*, it is frequently critical to determine antimicrobial susceptibility of all clinical isolates for best possible therapy of infected patients.¹⁴ Due to the restricted choice of antibiotics available for the treatment of Methicillin-resistant staphylococcal infections and the identified limits of Vancomycin and Clindamycin should be considered for the management of serious soft tissue infections with MRSA that are sensitive to Clindamycin.¹⁵

In our study, we have included 200 *S. aureus* isolates derived from pus 30 (41.09%), post-operative wound infection 13 (17.80%), blood samples were 16 (21.91%), Miscellaneous Samples were 10 (13.69%) and urine sample 4 (5.47%) from both outpatients and inpatients of Orthopaedic Department of our organization. The incidence of Methicillin resistance amongst all *S. aureus* isolates was found to be 36.5%. This difference data due to extended different geographical region. A comparable prevalence rate of 24, 34.6%, and 36.6% were also reported from Northern Bihar, and West Champaran Bihar^{16,17,18}

Although MRSA showed higher susceptibility to individual antibiotics when compared with others, we obtained high percentage of multidrug resistant MRSA from these specimens. Poddar CK, et al. from Bihar had reported 24% of

the MRSA isolated from clinical specimens to be multidrug resistant.¹⁶ Pappu RK, Poddar CK, et al. from Northern Bihar had reported a higher percentage of multidrug resistant MRSA.¹⁷ Rajak KC, Poddar CK, et al. from Bihar reported higher% of MRSA but from high risk patients admitted in burns and orthopedic units.¹⁸

In our study the inducible clindamycin resistant *S. aureus* isolates by detecting their antimicrobial susceptibility to various other antibiotics. It was found that all isolates with iMLS phenotype were 100%, susceptible to linezolid and vancomycin, moderate susceptibility (71,14%) to gentamicin, cefuroxime and least susceptibility to doxycycline, ciprofloxacin (23.81% and 20.95% respectively). This finding is also similar to other studies all the iMLS isolates were uniformly susceptible to linezolid and vancomycin.^{19,20,21}

CONCLUSION

In conclusion the importance of Isolation of MRSA patients and carriers in the hospitals, regular surveillance of hospital associated infections, including monitoring antibiotic sensitivity pattern and strict drug policy for antibiotics may be helpful for reducing the incidence of these infections. In our study vancomycin was the only antibiotic found to give uniform sensitivity (100%). When antimicrobials including, vancomycin is considered for treatment, choice inevitably requires the need for in vitro susceptibility testing of every isolate of MRSA in the clinical laboratories.

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