



Original Article

Antibiotic susceptibility pattern of staphylococcus aureus and methicillin-resistant staphylococcus aureus in a tertiary care hospital

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ABSTRACT

Background: Methicillin resistant Staphylococcus aureus has emerged as one of the most important nosocomial pathogens. It invokes a tremendous financial burden and enhanced morbidity and mortality due to difficult to treat systemic infections. Aim of this study was to determine antibiotic susceptibility pattern of Staphylococcus aureus and Methicillin resistant Staphylococcus aureus.

Materials and Methods: Different clinical specimens were collected and processed for routine culture and antibiotic sensitivity test by standard microbiology techniques.

Results: Out of 1173 samples received for microbiological examination, 100 were found to be S. aureus with 19% cases were Methicillin resistant Staphylococcus aureus (MRSA). Fourteen MRSA were found from inpatient and 5 were from outpatient. MRSA was found higher in female than male and maximum number (31.5%) was found in age group 0-10 years. Staphylococcus aureus was 100% sensitive to Vancomycin followed by Amikacin (90%), Gentamycin (83%), and tetracycline (81%). On urine isolates Nitrofurantoin (91.6%) was drug of choice. All the isolates were resistant to Penicillin G. In case of Methicillin resistant Staphylococcus aureus showed 100% sensitive to Vancomycin followed by Amikacin (84.2%), Tetracycline (63.1%), Ciprofloxacin (42%) and Gentamycin (36.8%). Among urine isolates Nitrofurantoin showed 87.5% sensitive followed by Norfloxacin (75%).

Conclusion: Methicillin resistant Staphylococcus aureus was found 19% of Staphylococcus aureus isolates. It was most common in females, hospitalized patients and young age group. Vancomycin seems to be drug of choice followed by Amikacin. It would be helpful to formulating and monitoring the antibiotic policy and ensure proper empiric treatment.

INTRODUCTION

Staphylococcus aureus (S. aureus) is important cause of community and hospital acquired infections. Infections

caused by Methicillin or Oxacillin resistant S. aureus (MRSA) are mainly nosocomial and are increasingly reported from many countries worldwide.¹ As MRSA strain are frequently resistant to many different classes of antimicrobial drugs, second- and third line antimicrobial resistance is a growing concern.² Methicillin was first introduced in human medicine in the 1960s for the treatment of infections caused by Penicillin's resistant S. aureus, but

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within a few years, MRSA emerged.³ Methicillin resistance in staphylococci is mediated by the *mecA* gene, which encodes for the penicillin-binding protein 2A (PBP2A) resulting in reduced affinity for the beta-lactam antibiotics including the penicillinase-resistant penicillin. MRSA has become a major hospital pathogen in human medicine.⁴

However, Vancomycin resistant *S. aureus* is not widely seen even though a low level resistance to Vancomycin is being reported.⁵ The risk factors which contribute to MRSA are excessive antibiotic usage, prolonged hospitalization, intravascular catheterization and hospitalization in intensive care unit.

The prevalence rate of MRSA is various in different countries such as 1-5% in Northern Europe, 5-30% in Southern Europe, 5-40 % in Asian countries and 10-50 % in USA and in UK. The problems of treating such infections are mainly due to the multi-drug resistant nature of MRSA. The prevalence of MRSA in Nepal is reported to be 15-69% from different tertiary care hospitals.⁶⁻⁸

The emergence of antibiotic resistant bacteria constitutes a major problem in antibiotic therapy. This could be attributed to unrestricted use of antibiotics in a particular environment. The present study aim was to know antibiotic sensitivity pattern of *Staphylococcus aureus* isolates in order to utilize the information obtained and formulate antibiotic policy and appropriate control measures.

MATERIALS AND METHODS

A prospective study was conducted at Microbiology Laboratory of Kist Medical College, Lalitpur during August 2011 to November 2011 included all patients both outpatient and inpatient. The research objective and methods were explained to the patients and informed consent was taken from each patient before collection of specimen.

In this study 1173 different clinical specimens (urine, blood, pus, sputum, swab, fluid/CSF and tips) were collected for routine culture and antibiotic susceptibility testing and were processed according to the standard microbiology laboratory techniques. The sample collected was evaluated in terms of its acceptability, proper labeling (full name, age, sex, serial number of the patient, date of collection).

The specimens were cultured on blood agar and MacConkey agar plates and incubated aerobically at 37°C for 24 hours. *Staphylococcus aureus* was identified by standard microbiology test such as colonies characteristics, gram staining reaction, biochemical tests such as catalase test, coagulase test and mannitol salt fermentation test. Antibiotic sensitivity of the isolates was performed according to the guidelines given by the NCCLS recommended Kirby-Bauer sensitivity testing method. The antibiotics which were used based on the standard protocol of the hospital

and the department policies. The antibiotic discs used for the susceptibility tests were from Hi-Media Laboratories Pvt. Limited. Different antibiotics used for *Staphylococcus aureus* are as follows: Penicillin (10 units), Ampicillin (10 µg), Vancomycin (30 µg), Amikacin (30 µg), Cotrimoxazole (1.25/23.75 µg), Ciprofloxacin (5 µg), Gentamycin (30 µg), Tetracyclin (30 µg), Erythromycin (15 µg), *Nalidixic Acid (30 µg), *Norfloxacin (10 µg), *Nitrofurantoin (300 µg), * used only for urine isolates. Screening of MRSA was performed according to the guidelines given by the Clinical and Laboratory Standards Institute 2011.

Data was analyzed by EPI-Info version 3.3.2, document version 8.08 updated Sept 2005 and presented by tables and diagrams.

RESULTS

Out of 1173 samples 328 (27.9%) were culture positive. Among them 46.3% were urine, 32.9% were blood, 11.5% were pus, 4.5% were sputum, 3.1% were swab, 0.25% were fluid/CSF and 1.5% were tips. Out of 328 cultures positive cases, prevalence of *S. aureus* were 100 (30.4%) and others were 228 (69.5%). Among 100 isolates of *S. aureus*, 19 were found to be Methicillin resistant *S. aureus*. The isolates of *S. aureus* and MRSA was higher in ward patient (54%) and (73.6%) than OPD (46%) and (26.3%) respectively. Gender wise distribution of *S. aureus* and Methicillin resistant *Staphylococcus aureus* was found higher in female than male. Age wise distribution of *S. aureus* and MRSA is shown in Table 1 in which *Staphylococcus aureus* was most prevalent in 51-60 years old group and MRSA in children (0-10 years). Antibiotic susceptibility pattern of both *S. aureus* and MRSA is shown in Table 2.

DISCUSSION

Despite the introduction of antimicrobial therapy and the recent improvements of medical services, MRSA is recognized as a major cause of nosocomial infections which result in significant morbidity and mortality rates. *S. aureus* is a very common cause of infection in hospitals and is most liable to infect new born babies, surgical patients, old and malnourished persons and patients with diabetes and other chronic diseases.⁹

A total of 1173 samples were processed, out of them 328 were positive for bacterial growth. In culture positive cases *S. aureus* accounted for 30.4% (100). The number of *S. aureus* isolated was high in ward (54%) than OPD (46%) and isolates were high in females than males. Out of 100 *S. aureus* isolates 19 isolates were found to be Methicillin resistant *S. aureus*. Out of that 8 MRSA were isolated from urine, 6 from blood and 5 from pus. In contrast to our study, Mahmood et al included 265 cases of which 102 (38.4%) were from sputum, 76 (28.6%) from IV catheters/ETT/suction tips etc 65 (24.5%) from pus/wound swabs,

Table 1: Age wise distribution of Staphylococcus aureus and Methicillin resistant S. aureus

Age group	Staphylococcus aureus			Methicillin resistant S. aureus		
	Male	Female	%	Male	Female	%
0-10	16	8	24%	5	1	31.5%
11-20	3	14	17%		5	26.3%
21-30	6	12	18%		3	15.7%
31-40	5	7	12%		2	10.5%
41-50		10	10%		2	10.5%
51-60	10	9	19%		1	5.2%

17 (6.4%) from blood and 5 (1.8%) from urine cultures.¹⁰ Another study conducted by Vaez et al rate of MRSA strains isolation in wound and sputum were higher than other samples with 42.9% and 40% respectively.¹¹ This is consistent with the reports from Tahniwale reported 19.5% of the total isolates of the S.aureus were MRSA.¹²

The patients between 0-10 age group showed the maximum positive growth for S. aureus (24%) followed by age group 51-60 (19%). The patients between 0-10 age group showed the maximum MRSA (31.5%), followed by age group 11-20 (26.3%). Age group 0-10 tends to have higher colonization rates, probably because of their frequent contact with respiratory secretions and may be due to sanitary reason. It is believed that their immunity is not properly developed at this stage to cope with bacterial infections hence they are vulnerable and easily infected especially when hospitalized. The older children have also been observed to be more active than adults during their interaction with their playmates

and while playing for hours, come in contact with various objects. In this process, they become a target to ubiquitous bacteria such as S. aureus. In contrast to this study Mahmood et al reported that majority of patients 170 (64.1%) were in age group 41-80 years.¹⁰

Finding of this study showed that S. aureus were found higher in female 60% than male 40% and MRSA were also found higher in female 73.6% than male 26.3%. Male and Female ratio of this distribution was 2:3. In contrast to this study MRSA predominance was observed in males 155 (58.5 %) than female 110 (41.5 %), Male to female ratio was 1:0.7.10 Another study performed by Khanal et al the rate was significantly higher among males (75%) than females (25%). MRSA infection was higher among patients above 30 years of age.¹³

Inpatients department showed maximum number of S. aureus (54%) and outpatient department showed (46%). Out of 19 MRSA (73.6%) were from inpatient department and (26.3%) were from outpatient department. This difference could be due to prolonged hospital stay, instrumentation and other invasive procedures and S.aureus is one of the major organisms causing hospital acquired infection. Study conducted by Sanjhana et al showed that (62.3%) Methicillin resistant S. aureus were isolated from inpatient departments and (37.7%) were from outpatients, which is comparable with our study.¹⁴

Antibiotic susceptibility pattern of S. aureus showed that Vancomycin was 100% sensitive, followed by Amikacin 90%, Gentamycin 83%, Tetracycline 81%, Ciprofloxacin

Table 2: Antibiotic susceptibility pattern of S. aureus and Methicillin resistant S. aureus

Antibiotics	Staphylococcus aureus			Methicillin resistant S. aureus		
	Sensitive	Intermediate	Resistance	Sensitive	Intermediate	Resistance
Penicillin	0 (0%)	0 (0%)	100 (100%)	0 (0%)	0 (0%)	19 (100%)
Ampicillin	22 (22%)	3 (3%)	75 (75%)	2 (10.5%)	3 (15.7%)	14 (73.6%)
Vancomycin	100 (100%)	0 (0%)	0 (0%)	19 (100%)	0 (0%)	0 (0%)
Amikacin	90 (90%)	6 (6%)	4 (4%)	16 (84.2%)	0 (0%)	3 (15.7%)
Cotrimoxazole	58 (58%)	13 (13%)	29 (29%)	6 (31.5%)	1 (5.2%)	12 (63.1%)
Ciprofloxacin	59 (59%)	22 (22%)	19 (19%)	8 (42.1%)	2 (10.5%)	9 (47.3%)
Gentamycin	83 (83%)	2 (2%)	15 (15%)	7 (36.8%)	1 (5.2%)	11 (57.8%)
Tetracyclin	81 (81%)	5 (5%)	14 (14%)	12 (63.1%)	2 (10.5%)	5 (26.3%)
Erythromycin	31 (31%)	29 (29%)	40 (40%)	1 (5.2%)	1 (5.2%)	17 (89.4%)
*Nalidixic Acid	3 (12.5%)	0 (0%)	21 (87.5%)	0 (0%)	0 (0%)	8 (100%)
*Norfloxacin	12 (50%)	0 (0%)	12 (50%)	6 (75%)	0 (0%)	2 (25%)
*Nitrofurantoin	22 (91.6%)	0 (0%)	2 (8.3%)	7 (87.5%)	1 (12.5%)	0 (0%)

*Antibiotic used only in urine sample

59%, Cotrimoxazole 58%, Erythromycin 31%, Ampicillin 22% and all the isolates were resistant to Penicillin G. Nitrofurantoin, Norfloxacin and Nalidixic acid were used for urine isolates and showed sensitivity of 91.6%, 50% and 12.5% respectively. Study conducted by Khanal et al showed that resistance pattern of *S. aureus* to different antibiotics, Erythromycin 75.3%, Ciprofloxacin 70% and Vancomycin 21%. That study indicates the emergence of Vancomycin resistant *S. aureus* (VRSA).¹³ Similar study performed by Baral et al *S. aureus* showed susceptible to Tetracycline (94.3%), Erythromycin (89%), Ciprofloxacin (88.3%) and Gentamicin (78%). Sixty four percent of isolates were found to be resistant to Co-trimoxazole and 26% were Methicillin resistant (MRSA). Nearly half (52.6%) of *S. aureus* showed resistance to Penicillin. All the isolates were susceptible to Vancomycin.¹⁶ In study done by Vaez et al resistance pattern to Penicillin, Ampicillin, Nalidixic acid, Erythromycin, Ciprofloxacin, Gentamicin, Tetracycline and Vancomycin was 97.3%, 97.3%, 53%, 29.2%, 22.7%, 20.5%, 31.4% and 0% respectively.¹¹ These studies are consistent with our finding.

Antibiotic susceptibility pattern of Methicillin resistant *S. aureus* showed that Vancomycin was 100% sensitive followed by Amikacin 84.2%, Tetracycline 63.1%, Ciprofloxacin 42.1%, Gentamycin 36.8%, Cotrimoxazole 31.5%, Ampicillin 10.5%, and Erythromycin 5.2%. In urine isolates Nitrofurantoin showed 87.5% sensitive followed by Norfloxacin 75% and all the isolates are resistant to Nalidixic acid. This is consistent with the reports from Mahmood et al antimicrobial sensitivity pattern of MRSA showed 28.7% to Ciprofloxacin, 37.5% to Gentamycin, 27.5% to Erythromycin, 8% to Penicillin and 100% to Vancomycin.¹⁰

CONCLUSION

In this study Methicillin resistant *S. aureus* was found 19% of the *Staphylococcus aureus* positive cases. MRSA was common in female, admitted patients and lower age group. For treatment of *S. aureus* infection Vancomycin (100%) was the drug of choice followed by Amikacin (90%), Gentamycin (83%), and Tetracycline (81%). On urine isolates Nitrofurantoin (91.6%) was drug of choice. All the isolates were resistant to Penicillin G. In case of MRSA Vancomycin (100%) seems to be drug of choice followed by Amikacin (84.2%). This study highlights the need for continuous surveillance of antibiotic sensitivity pattern of *S. aureus* and (MRSA). It would be helpful to formulating and monitoring the antibiotic policy and ensure proper empiric treatment.

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