Original Article

Prevalence of Methicillin Resistant *Staphylococcus aureus* in Patients visiting a Tertiary Care Hospital, Western Nepal

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ABSTRACT

Introduction: *Staphylococcus aureus* is most commonly isolated organism from different clinical specimens in hospitals. Methicillin resistant *Staphylococcus aureus* (MRSA) is a global health challenge nowadays creating problem in antibiotic therapy. This study was conducted to find out the prevalence of MRSA at a tertiary care hospital, western part of Nepal. **Materials and Methods:** Hospital based cross-sectional study was conducted at Department of Microbiology, Universal college of Medical Sciences and Teaching Hospital (UCMS-TH), for one year from January to December 2017. A total of 4841 different clinical specimens (Urine 2376, Pus/swab 1253, blood 665, CSF 175, body fluid 220 and sputum 152) were collected from the patients aged between 0 to 96 years who attended the hospital for treatment. Culture and bacterial identification were done by using standard microbiological guidelines. Antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method following clinical and laboratory standards institute (CLSI) guidelines. MRSA was identified by using cefoxitin (30 µg) discs. **Results:** From the total of 4841specimens processed in the laboratory, Growth was found in 1267 (26.2%) of samples, (Urine 519/1267, Pus/swab 531/1267, Blood 136/1267, CSF 7/1267, Body fluids 15/1267, Sputum 58/1267). Among 1267 different species of isolated organisms, *S. aureus* was 217(17.1%). Out of total 217 (17.1%) *S. aureus* isolated from various clinical specimens, total 95 (43.8%) were found to be MRSA and 122 (56.2%) were Methicillin sensitive *Staphylococcus aureus* (MSSA). The isolated organism showed high rate of resistance to commonly used antibiotics. **Conclusion:** This study demonstrates high prevalence MRSA in our hospital. The isolated organism showed high rate of resistance to commonly used antibiotics. Regular surveillance of hospital associated infection including monitoring antibiotic sensitivity pattern of MRSA is mandatory to control the spread in the hospital. A strict drug policy is very importance in every health care settings otherwise the threat will increase and even vancomycin and other glycopeptides resistant strains may emerge.

Key words: *Staphylococcus aureus*, MRSA, Nepal

INTRODUCTION:

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first reported in the early 1960s in the United Kingdom. These are strains of *S. aureus* that through the process of natural selection developed resistance to all available penicillins and other β-lactam antimicrobial drugs.[1] Over the next 4 decades, MRSA spread worldwide and became endemic in hospitals in many countries.[2] In the 1990s, community associated MRSA emerged as an epidemic of skin and soft-tissue infections in patients without any prior healthcare contact.[3,4] MRSA is a major public health problem worldwide, causing significant morbidity and mortality and elevated health care costs.[5,6]

The common sources of these infections are human patients and carriers.[7] The presence of mecA gene located on cassette chromosome in *S. aureus* (SCCmec) is responsible for methicillin resistance.[8] This gene encodes penicillin binding protein 2A (PBP2A) which has a low affinity for methicillin. The Cefoxitin (30 µg) disk is used to detect MRSA by the disk diffusion method. *S. aureus* that are mecA positive should be reported as resistant to oxacillin and other β-lactam antibiotics.[9] Some large outbreaks have been reported from different parts of the world, where it had caused severe infections including septicemia, endocarditis and meningitis.[10] Studies done in different hospitals of Nepal showed high rate of MRSA.[11-14] This study was done to find the prevalence of MRSA in Universal college of Medical sciences and Teaching Hospital (UCMS-TH) Bhairahawa, Nepal and to determine antimicrobial

susceptibility profiles of the isolated organisms.

MATERIALS AND METHODS

A longitudinal, hospital-based study was conducted at Department of Microbiology, UCMS-TH, Bhairahawa, Nepal over a period of one year from January to December 2017. A total of 4841 different clinical specimens (Urine 2376, Pus/swab 1253, blood 665, CSF 175, body fluid 220 and sputum 152) were collected from the patients aged between 0 to 96 years who attended the hospital for treatment.

Sample collection

The samples were collected in sterile containers by clinicians using aseptic technique and transported to the laboratory without delay. All samples were processed immediately.

Culture and bacterial identification

Samples collected in the laboratory for culture and sensitivity were inoculated into Chocolate agar (CHA) blood agar (BA), MacConkey's media (MA), Brain heart infusion (BHI) broth (for blood sample), (Hi Media Laboratories Pvt. Limited, India). Selective media for *S. aureus* was not used. *S. aureus* was identified by standard microbiological techniques.[15]

Antibiotic susceptibility testing

Antibiotic susceptibility tests of the *S. aureus* isolates were performed by modified Kirby-Bauer disk diffusion method in compliance with Clinical and Laboratory Standards Institute (CLSI) guidelines using Mueller-Hinton agar standard media. The inhibition zone standards for antimicrobial susceptibility were considered from tables for interpretative zone diameters of CLSI.[16] Antibiotic disks (Hi Media Laboratories, Pvt. Limited, India) used were: penicillin G (10U), ciprofloxacin (5µg), erythromycin (15µg), co-trimoxazole (25µg), gentamicin (10µg), amikacin (30 µg), ceftriaxone (30µg), cefoxitin (30µg), vancomycin (30µg), linezolid (30µg).

Identification of methicillin resistant *Staphylococcus aureus* (MRSA) strains

MRSA was identified by using cefoxitin (30 µg) discs. Muller Hinton agar Plates were inoculated with test organisms and cefoxitin (30 µg) discs were inserted in the plate and incubated at 37°C. Plates containing cefoxitin disc were read following a 24-hour incubation period. The diameter of the zone of inhibition (ZOI) of growth was recorded and interpreted as susceptible or resistant according to the criteria of CLSI. *S. aureus* isolates were deemed methicillin resistant when the ZOI was ≤21 mm with the cefoxitin disc.[17]

Quality Control

All media and reagents used in this study were tested and verified for sterility and performance. *S. aureus* ATCC 25923 was used as a control organism for antibiotic sensitivity testing. For MRSA detection, *S. aureus* ATCC 25923 and ATCC 43300 were used as negative and positive controls respectively.

Data analysis

All the data from cases were entered in MS Excel (Microsoft office 2007) and then analyzed by statistical package for social sciences (SPSS) for window version; SPSS 20 Inc, Chicago IL. All the data were expressed in the term of percentage and frequency.

Ethical Consideration

This study was approved by Institutional Review committee of Universal College of Medical Sciences, UCMS, Bhairahawa, Nepal.

RESULTS

A total of 4841 samples (Urine 2376, Pus/swab 1253, blood 665, CSF 175, body fluid 220 and sputum 152) from patients attending UCMS-TH for treatment were collected and analyzed. From the total of 4841samples, Growth was found in 1267 (26.2%) of samples, (Urine 519/1267, Pus/swab 531/1267, Blood 136/1267, CSF 7/1267, Body fluids 15/1267, Sputum 58/1267). Among 1267 different species of isolated organisms, *S. aureus* was 217(17.1%).

Out of total 217 (17.1%) *S. aureus* isolated from various clinical specimens, total 95 (43.8%) were found to be MRSA and 122 (56.2%) were Methicillin sensitive

Staphylococcus aureus (MSSA) (Figure 1).

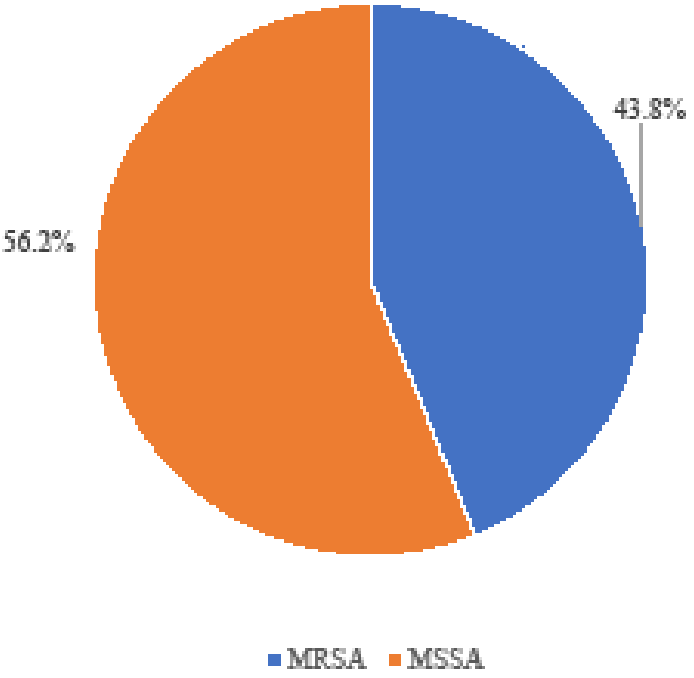

Figure 1: Prevalence of MRSA

Out of the total of 217 strains of *S. aureus* isolated, 144 (66.3%) was from pus/swab sample followed by 34 (15.7%) from blood, 32 (14.7%) from urine and others. In total isolated *S. aureus*, 95 were found to be MRSA (43.8%). The maximum isolation of MRSA was from pus and swab 66 (69.5%) followed by others. The number and percentage of isolated of *S. aureus* and MRSA from different clinical specimens is shown in (Table 1).

Table 1. Number and Percentage of isolated *S. aureus* and MRSA from different clinical samples

Clinical Samples	Growth rate in total sample	*S.aureus* isolated	MRSA detected
Pus/ Swab	531 (41.9%)	144 (66.3%)	66 (69.5%)
Urine	519 (41%)	32 (14.7%)	11 (11.6%)
Blood	136 (10.7%)	34 (15.7%)	14 (14.7%)
Sputum/ Endotracheal tube	58 (4.6%)	6 (2.8%)	4 (4.2%)
Body fliuds	15 (1.2%)	1 (0.5%)	0 (0)
CSF	7 (0.6%)	0 (0)	0 (0)
Total	1267 (100%)	217 (100%)	95 (100%)

The antibiogram of the *Staphylococcus aureus* isolated in culture is shown in Table 2. Linezolid and vancomycin retained (100%) susceptibility. Most of the isolates (91.7%) were resistant to penicillin, and most of them were resistant to other commonly used antibiotics. By using cefoxitin disk (30ug), (43.8%) of the isolates were detected as MRSA.

Table 2. Antibiogram of S. aureus (n=217)

Antimicrobial agents	Resistance isolates (%)
Penicillin	199 (91.7%)
Cotrimoxazole	114 (52.5%)
Gentamycin	99 (45.6%)
Amikacin	67 (30.9%)
Cefoxitin	95 (43.8%)
Ceftriaxone	149 (68.7%)
Levofloxacin	111 (51.1%)
Ciprofloxacin	139 (64%)
Erythromycin	132 (60.8%)
Vancomycin	0 (0)
Linezolid	0 (0)

DISCUSSION:

The prevalence of *S. aureus* in UCMS-TH Bhairahawa was (17.1%). It is one of the most successful and adaptable human pathogens causing both nosocomial and community infections.[18] Among the isolated *S. aureus* from various clinical samples, the prevalence of MRSA infection in our study was found to be (43.8 %), which is in accordance with the reports by Ansari et al in Chitwan (43.1%), Mishra et al in Kathmandu (42.4%) and Raut et al in Palpa (43.8%).[11-13] However, Varying prevalence of MRSA has been reported from different parts of Nepal. Study done by Kumari et al. in Dharan reported (26.1%), Khanal et al in Chitwan reported (68%), Shakya et al in Birgunj reported (57.1%) [14,19,20] The high prevalence of *S. aureus* infection may be because it is an endogenous source of infections.[21]

S. aureus may gain access to the epidermis through cracks in the skin, abrasions, cuts, burns, surgical incisions and intravenous catheters causing wide spectrum of infections, from localized skin lesions such as abscesses, folliculitis to deep seated infections.[11] In the present study, out of total 217 isolated. *S. aureus*

from different clinical specimens' majority 144 (66.3%) were isolated from pus/swab sample. This result shows *S. aureus* is a major pathogen in abscess formation.

This study demonstrated high rate of resistance to commonly used antibiotics for the treatment of infections caused by *S. aureus*. A high proportion of isolates (91.7%) were resistant to penicillin in this study. Among the isolated organisms, Vancomycin and Linezolid were the most effective antibiotics. *S. aureus* is one of the most successful and adaptable human pathogens for its property in acquiring antibiotic resistance mechanisms and pathogenic determinants, leading to its emergence in both nosocomial and community infections.[18]

Erythromycin has been used extensively for the treatment of both minor and more serious staphylococcal infections. But today it's use is increasingly limited due to increasing resistance, which poses a great therapeutic challenge. More than (60%) of our isolates were resistant to erythromycin, compared to previous similar studies in Nepal which have round resistance rates of (7.1%) in 2010, (11%) in 2011, (63.6%) in 2013. [20,22,12] Likewise, trimethoprim-sulfamethoxazole (co-trimoxazole) can be an alternative treatment choice, particularly for non-multi-resistant MRSA infections, although emergence of resistance has been previously observed. This may be due to excessive use of this drug for many other infections and over-the-counter availability of antimicrobials in the developing world for the treatment of many other infections. Cotrimoxazole resistance was found in (52.5%) of our isolates, compared with (42.96%) in 2009 [23], (12.5%) in 2010 [24], (64%) in 2011 [22], and (72.7%) in 2013 [12] in Nepal. Because of low cost and easy availability in Nepal, ciprofloxacin has been extensively used and it has now created bad consequences. We identified resistance rate of (64%) towards ciprofloxacin which is much higher than previous studies (26%) in 2009 [25] , (12%) in 2011 [22]. The same trend is seen with gentamicin, (45.6%) of our isolates being resistant compared with (46.98%) in 2009 [23], (32.73%) in 2010 [24], (11%) in 2011 [22], and (54.5%) in 2013.[12] The rate of resistance to amikacin is low as compared to other drugs which is (30.9%) but higher than other studies done at different places of Nepal.[11-14]

All isolates were susceptible to Vancomycin and Linezolid which is consistent with previous studies .[12, 14, 22, 24, 25] From this we can confirm that glycopeptides should be used as empiric therapy for serious staphylococcal infections while waiting for susceptibility testing results to come through.[26]

Rates of resistance in *S. aureus* is increasing in Nepal due to many factors. Nepal is a developing country there is no specific rules for the purchase and use of antibiotics. People can use antibiotics without prescription. Many antibiotics are prescribed without culture and sensitivity due to lack of laboratory facilities in most of the areas. Irrational use of antibiotics is a major cause for drug resistant. In addition, infection control policies are yet to be instituted properly in most of the hospitals and medical institutions of Nepal. For the reduction of MRSA infection, regular surveillance of hospital associated infections and antibiotic sensitivity pattern of MRSA is very important. Definite antibiotic policy should be formulated and should be implemented properly for the reduction of the incidence of MRSA infection. In addition, infection control program should be conducted effectively in all health care centers and effective trainings should be given to the healthcare workers regularly for the control of hospital acquired infections.[27-29,11]

Conclusion

This study demonstrates high prevalence MRSA in our hospital. The isolated organism showed high rate of resistance to commonly used antibiotics. Regular surveillance of hospital associated infection including monitoring antibiotic sensitivity pattern of MRSA is mandatory to control the spread in the hospital. A strict drug policy is very importance in every health care settings otherwise the threat will increase and even vancomycin and other glycopeptides resistant strains may emerge. Knowledge about MRSA and carrier status should be cleared among the health care professionals of every hospital and control measures need to be implemented consistently in order to reduce the burden of MRSA infection in the hospital environment. A longitudinal surveillance of MRSA and its antimicrobial susceptibility profile should be done in Nepal for the epidemiological mapping of the infections caused by the organism.

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