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Prevalence and Antibiogram of Methicillin Resistant Staphylococcus Aureus (MRSA) in a Tertiary Care Hospital in Central Nepal.

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ABSTRACT

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is one of the major causes of nosocomial infections worldwide and is responsible for significant morbidity and mortality of the patients. It is usually resistant to many antibiotics and therefore diseases caused by MRSA are more difficult and expensive to treat than Methicillin-Sensitive *Staphylococcus aureus* (MSSA). The aim of this present study was to determine the prevalence of MRSA infections and their antimicrobial susceptibility pattern in our hospital located in central Nepal. Clinical specimens received from October 2016 to March 2017 in bacteriology laboratory of Microbiology, College of Medical Sciences-Teaching Hospital. The specimens were processed and identified by standard laboratory procedures. The antibiotic susceptibility testing of all included *Staphylococcal* strains was performed by modified Kirby Bauer disc diffusion method. During this study period, a total of 173 isolates of *Staphylococcus* (S) *aureus* were studied and 75 (43.35%) were found to be MRSA. In our study maximum percentage of *S. aureus* (49.13%) and MRSA isolation (49.33%) were from pus and wound swab. The majority of the samples were obtained from surgery (53.19%) and ICU (19.15%). In the study penicillin, oxacillin resistance was observed to be 100%, 43.35% respectively against the organism. About 50–55% of MRSA were resistant to erythromycin, gentamicin and ciprofloxacillin while less than 30% were resistant to levofloxacillin. However, this present study also indicates the emergence of Vancomycin Resistant *S. aureus* (VRSA)-9.25%. To reduce the prevalence of MRSA and its spread to community as well, the regular surveillance of hospital-associated infections, isolation of patients who carry MRSA, monitoring of antimicrobial susceptibility pattern and formulation of a definite antibiotic policy may be helpful.

Introduction

In the 21st century, the emergence of hospital acquired infections including methicillin-resistant *S. aureus* (MRSA) and extended spectrum beta lactamases (ESBL), is considered to be one of the most important threats to human health. The medical importance of *Staphylococcus aureus* (*S. aureus*) has been heightened by its ability to adapt rapidly to the selective pressure of antibiotics and the resultant emergence and spread of MRSA. *S. aureus* is a leading cause of hospital and community-associated infections[(1)]. In the hospital, *S. aureus* is the most frequent cause of lower respiratory tract, surgical and cardiovascular infections. It is also responsible for health care-associated pneumonia and bloodstream infections[(2)]. Historically, β-lactam antibiotics have exhibited potent activity against *S. aureus*, which along with good safety profiles make them the best agents of choice for the treatment of *Staphyloccocal* infections. Methicillin was introduced by Beecham in 1959 but approximately two years after the introduction of methicillin in 1961, the first *S. aureus* isolates resistant to methicillin was reported in the UK[(3,4)]. Later on, Hospital-associated (HA) MRSA and community-associated (CA) MRSA infections constitute a major burden on society world-wide[(5)].

In MRSA, the *mecA* gene confers production of an altered PBP, PBP2a, to which beta-lactam antimicrobials are not able to bind, and therefore cannot disrupt peptidoglycan synthesis, enabling the growth and survival of MRSA[(6)]. Resistance to beta-lactam antibiotics, which are the most widely used group of antibiotics, makes MRSA infections very difficult to treat and only very few alternative drugs are currently available for treatment.

Vancomycin is the first-line drug to treat the severe MRSA infections[(7)]. It is however less efficient, requires intravenous administration and resistance has already been reported in the form of vancomycin-resistant *Staphylococcus aureus* (VRSA)[(8,9)]. Prolonged hospital stay and indiscriminate use of antibiotics increase the chance of emergence and spread of MRSA. The rapid and accurate identification of MRSA in clinical specimens has important implications for the therapy and management of both colonized and infected patients.

The increasing prevalence of MRSA worldwide is a growing public health concern. Nepal has not been spared from the increase in the number of MRSA cases. The reported prevalence of MRSA in Nepal shows an increasing trend; 29.1% in 1990[(10)] and 76.0% in 2003[(11)] .

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There is limited study addressing MRSA prevalence has been conducted in the central region of Nepal which has relatively better health care facilities, offers easier access to antibiotics and receive many patients from this region and neighboring states of India. Therefore, the current study estimates the percentage of MRSA strains and investigates their antibiotic resistance profiles in this central region of Nepal.

Materials and Methods

This study was a laboratory-based retrospective cross sectional study on MRSA isolates. The study was conducted in bacteriology laboratory of department of Microbiology of tertiary care hospital, College of Medical Sciences Teaching Hospital (COMS), located on Bharatpur of Chitwan district of Nepal from October, 2016 to March, 2017. One sample from one patient was inclusion criteria of study data, second sample from other site of same patient and coagulase negative *Staphylococcus* were excluded from the study data. This study consists of 173 strains of all methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) isolates collected from various clinical samples from different patients visiting OPD and admitted to COMS, Bharatpur.

These organisms were obtained from various clinical samples including pus, sputum, urine, blood, genital specimens (high vaginal swab, urethral discharge and semen), body fluids (synovial fluid, cerebrospinal fluid, ascitic fluid, pleural fluid, peritoneal fluid), devices (urinary catheter, suction catheter, endotracheal tube, tracheostomy tube) and respiratory specimens submitted to Bacteriology Laboratory of the hospital.

In Microbiology laboratory, specimens were inoculated onto Blood agar and MacConkey agar (Hi-Media, India). Urine specimens were inoculated onto Cysteine Lactose Electrolyte Deficient agar, CLED (Hi-Media, India). Samples for Blood culture were inoculated in Brain Heart Infusion (BHI) broth and subcultured on 24 and 72 hours on blood agar and MacConkey agar. *S. aureus* strains were identified based on standard isolation procedures[12,13]. (Gram stain, catalase test, mannitol fermentation, slide coagulase and tube coagulase test).

Mueller-Hinton agar (MHA) plates were overlaid with the saline suspension of a strain (turbidity = 0.5 McFarland standard) and the antibiotic susceptibility pattern of all the strains was determined by modified Kirby Bauer disc diffusion method against the following antibiotics: penicillin (10 units), oxacillin (1µg), gentamicin (10µg), amikacin (30µg), erythromycin (15µg), meropenem (10µg), co-trimoxazole (25µg), ampicillin/sulbactum (10/10µg), ciprofloxacin (5µg), levofloxacin (5µg),vancomycin (30µg), amoxyclav (30µg) and cefotaxime (30µg). These antibiotics were obtained from Hi-Media Laboratories Pvt. Ltd. Methicillin resistance was confirmed by agar screen test using Mueller-Hinton agar plate supplemented with 4% NaCl and oxacillin discs (1µg) and incubated for 24-48 hours at 37°C. The interpretive criteria of the CLSI for *Staphylococcus aureus* was used to establish the antibiotic susceptibility of the isolates[14]. Methicillin sensitive *S. aureus* (MSSA) ATCC 25923 and methicillin resistant *S. aureus* (MRSA) ATCC 43300 were used to verify the quality and accuracy of testing procedures[14,15].

Results

A total of 173 *S. aureus* isolates were collected between October 2016 and March 2017.

Out of the total 173 strains of *S. aureus* examined, 75 (43.35%) were found to be Methicillin-resistant *S. aureus* (MRSA).

The maximum isolation of MRSA was from pus and sputum. The number and percentage of isolation of MRSA from different clinical specimens are given in table 1. The MRSA isolates 62.67% was from the inpatient departments. The majority of the samples were obtained from surgery (53.19%) and ICU (19.15%) as shown in table 2.

Result of antibiotic sensitivity test showed the resistance pattern of *S. aureus* to different antibiotics including erythromycin-57.22%, ciprofloxacin-52.6% and vancomycin-9.25% as shown in table-3. This study also indicates the emergence of Vancomycin Resistant *S. aureus* (VRSA).

Table 1. Frequency of Staphylococcus *aureus* and MRSA in various clinical samples.

Clinical samples	*S.aureus* (Total, n=173)	MRSA (Total, n=75)
Pus (swab/aspirate)	85 (49.13%)	37 (49.33%)
Blood	8 (4.62%)	2 (2.67%)
Body fluids (synovial fluids/ pleural fluids etc)	10 (5.78%)	3 (4.00%)
Sputum/ throat swab	29 (16.76%)	14 (18.67%)
Urine	20 (11.56%)	7 (9.33%)
Genital specimens(high vaginal swab, urethral discharge, semen)	19 (10.98%)	12 (16.00%)
CSF	02 (1.16%)	00
Total	**173**	**75**

Table 2. Distribution of MRSA in Inpatient and Outpatient departments.

Departments	Inpatients (47)	Outpatients (28)
Surgery	25 (53.19%)	5 (17.86 %)
Orthopedics	5 (10.64%)	00
ENT	2 (4.25%)	7 (25%)
Medicine	2 (4.25%)	6 (21.43%)
ICU	7 (14.89%)	00
Obs/Gyne	6 (12.76%)	10 (35.71%)
Total	**47**	**28**

Table 3. Shows antibiotic susceptibility patterns of Methicillin sensitive and resistant *S. aureus.*

S. No.	Antibiotics	Sensitive	Resistant
1	Penicillin-G (10 units)	00	173 (100%)
2	Oxacillin (1µg)	98 (56.65%)	75 (43.35%)
3	Gentamicin (10µg)	82 (47.40%)	91 (52.60%)
4	Amikacin (30µg)	80 (46.24%)	93 (53.75%)
5	Meropenem (10µg)	113 (65.31%)	60 (34.68%)
6	Ampicillin/Sulbactum ((10/10µg)	78 (45.09%)	95 (54.91%)
7	Amoxyclav (30µg)	86 (49.71%)	87 (50.23%)
8	Co-trimoxazole (25µg)	35 (20.23%)	138 (79.77%)
9	Ciprofloxacillin (5µg)	82 (47.40%)	91 (52.60%)
10	Levofloxacillin (5µg)	147 (84.97%)	26 (15.03%)
11	Erythromycin (15µg)	74 (42.77%)	99 (57.22%)
12	Cefotaxim (30µg)	110 (63.58%)	63 (36.42%)
13	Vancomycin (30µg)	157 (90.75%)	16 (9.25%)

Discussion

Methicillin resistant *Staphylococcus aureus* (MRSA) is a significant cause of health care and community-associated infections. MRSA is a major nosocomial isolate in hospitals which is associated with significant morbidity and mortality [16,17]. This extreme morbidity and mortality is due largely to the fact that many *S. aureus* strains carry genes and plasmids

that provide resistance to a variety of antibiotics, including the most efficient and widely used anti-*Staphylococcal* drugs.

The prevalence rate of MRSA in our study was found to be 43.35% which was in accordance with the study on MRSA in Nepal; Tribhuvan university (TU)-teaching hospital[18] revealed its prevalence to be 44.9%. Similarly the other study from same tertiary care hospital on MRSA among patient in Chitwan[19] showed its prevalence to be 39.6%. On the contrary, some of the reports showed an alarmingly high incidence of MRSA infection ranging from 62% to 80% [20,21,22]. Similar other study on MRSA in Pokhara[23] found to be 56.1%, in Birganj[24] 57.1%. However, low prevalence rate of MRSA were also reported from international as well as national scenario[25-28].

The reason for variation in the percentage of isolation of MRSA might be due to indiscriminate use of antibiotics, prolonged hospital stay, lack of awareness and failure to observe simple yet effective infection control precautions like strict patient isolation and frequent hand washing by health care personnel. The distribution of MRSA also varies according to factors such as population, areas studied (different institutions in a given area), the use of different culture techniques and different interpretation guidelines.

In our study maximum percentage of *S. aureus* (49.13%) and MRSA isolation (49.33%) were from pus and wound swab which was similar to the findings of a study from Nepal (Bharatpur and Dharan), Pakistan, India [19,26,21,29]. This may be attributed to the role of the organism as cause of pyogenic infections. Our findings of 62.67% MRSA from inpatients and 37.33% from outpatients departments are similar with those of a national study[11] that reports 70% and 30% prevalence in the two settings, respectively.

Determination of antimicrobial susceptibility of bacterial pathogens using classical phenotypic tests in clinical microbiology is important in the therapeutic treatment of patients which depends on assessing the susceptibilities of the bacteria[30]. Hospital acquired MRSA demonstrated resistance to most antimicrobials used in this study with rates ranging from 50.23% to100%. It is generally expected that MRSA resistance rates to penicillin are very high[31,32]. The most important resistance mechanism to penicillin is production of the beta-lactamase which inactivates penicillin by hydrolysing the beta-lactam ring. This research showed that beta-lactam antibiotics like penicillin, oxacillin are ineffective drugs against *S. aureus*. In the present study penicillin, oxacillin resistance was observed to be 100%, 43.35% respectively against the organism. This corroborates with the finding of Anupurba *et al*[33] who reported penicillin and cephalexin resistance were 100% and 88.7% respectively.

The choice of antibiotic for therapy of MRSA infections is usually complicated. Therefore, an important implication of the antimicrobial susceptibility results from the current study is their interpretation in relation to treatment of MRSA infections. For example, co-trimoxazole and a combination of co-trimoxazole and other drugs are among the currently available treatment options for MRSA, especially in the community [34,35].

However, where resistance to co-trimoxazole is high, this may not be viable. In this study resistance rate of erythromycin is 57.22%, co-trimoxazole is 79.77%, gentamicin is 52.60%. Our findings are comparable to those of a study done in Taiwan[17] in which resistance rates of erythromycin was higher 94.9%, co-trimoxazole was about to same 71.8% and those of gentamicin was also higher 78.2%.

Another potential antibiotic in the treatment of MRSA infections is ciprofloxacin. Ciprofloxacin and other quinolone antibiotics have been proposed as possible alternatives to parenteral vancomycin therapy on the basis of several *in vitro* and *in vivo* animal model data[36]. The resistance to quinolone-ciprofloxacin and levofloxacin was found to be 52.60% and 15.03% in our study. Our findings are comparable to those of a study done in India[36] in which resistance rates of ciprofloxacin and levofloxacin were 57.6% and 30.4% respectively. Notably, some studies from Spain [37,38] showed higher (greater than 90%) resistance rates to ciprofloxacin. Although this result differs from those of Kapatamoyo *et al*[39] in 2010, who found very low ciprofloxacin resistances rates. Further, quinolones are relatively cheaper and easily available as over-the-counter drugs in Nepal. Such ciprofloxacin resistance rates in healthcare settings may render ciprofloxacin not to be useful as a first-line antibiotic[36]. Resistance to ciprofloxacin is usually due to spontaneous mutations in the gyrase and topoisomerase genes[40,41]. Moreover, it has been reported that ciprofloxacin resistant MRSA isolates tend to show increased resistance to other antibiotics, including aminoglycosides[42]. Further studies on the susceptibility of MRSA to ciprofloxacin and other quinolones in our setting are recommended. The resistance for cephotaxime and meropenam were 36.42% and 34.68% respectively (β- lactam resistance). This is probably due to the indiscriminate and empirical use of these drugs.

All clinical isolates of this study have shown that only 90.75% of *S. aureus* were susceptible to vancomycin where as sensitivity to other drugs was also poor. This indicates that the emergence and spread of resistance to vancomycin are a threat to the already challenging therapy of MRSA and raise an alarming situation to the clinicians in hospital as well as in community. 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[43] which we have used in this study for MRSA detection. Similarly isolation of VRSA may be due to lab markers of severe/ chronic diseases (haematocrit, haemoglobin, leukocyte count, platelet count and serum albumin) among cases during sample collection[44]. 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.

Conclusion

Report of this study indicates necessity of regular surveillance of MRSA and its antibiotic resistance profiles to formulate definite antibiotics policy to reduce the incidence of MRSA and multidrug resistant bacterial infections as well. The effective hospital infection control program and guidelines should be strictly implemented and followed so as to enable the clinicians to deliver better and proper health care to the patients.

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