Lack of regular surveillance within hospital premises, ineffective monitoring of antimicrobial susceptibility pattern of Methicillin-Resistant \textit{S. aureus} (MRSA), lack of formulation of definite antimicrobial policy, ineffective hospital decontamination procedures and unhygienic practices have led to spread of antibiotic resistant \textit{S. aureus} strains within wards and to the community. The morbidity and the mortality have increased proportionately with the acquisition of antibiotic resistant by MRSA. Therefore, the present study was conducted to determine the prevalence of MRSA from upper respiratory tract infection and to find out its antibiotic susceptibility pattern. Nasal swab were collected from OPD and ward patients in a clean, sterile and dry test tube and processed by standard microbiological techniques and antibiotic susceptibility testing (AST) was done by modified Kirby Bauer disc diffusion method. MRSA strains were screened by testing with cefoxitin dics having zone of inhibition less than 21 mm. The prevalence of MRSA was 28\% and found to be higher in ward with 70.37\% than OPD patients (29.62\%) and was statistically significant (p=0.001). The MRSA isolates showed highly resistant towards both pencillin, ampicillin with 92.60\%, ciprofloxacin and erythromycin with 55.56\% and 48.15\% repectively whereas greatly sensitive towards both vancomycin and tetracycline with 85.19\%. MRSA infection is still one of the most threatening infections in the hospitals of Nepal and globally such infections are difficult to treat which requires regular survillance and monitoring AST of MRSA.

\textbf{Key words:} Antibiotic Resistance, Methicillin Resistant \textit{Staphylococcus aureus} (MRSA), Upper Respiratory Tract Infection (URTI), Significant

\section*{INTRODUCTION}

The trend of multidrug resistance in \textit{S. aureus} is particularly increasing due to the severity and diversity of diseases caused by this pathogen which has been noticed as one of the supreme microbial threats of the 21\textsuperscript{st} century [Waldvogel FA, 2000; Smolinski MS et al, 2003]. Resistance of numerous bacterial pathogens to many antibiotics continues to increase globally. Frequencies, pattern, and distributions of resistant bacteria vary significantly with geographical regions and often reflect the practice patterns of antibiotics [Yadav K
and Prakash S, 2015]. *S. aureus* is common for both endemic and epidemic infections acquired in hospitals which results in substantial morbidity and mortality [Geffers C et al, 2004]. It is the most frequent human pathogen which is responsible for upper respiratory tract infection, impetigo, folliculitis, furuncle, wound infections, osteomyelitis, bacteremia with metastatic complications, and food poisoning, toxic shock syndrome, scaled skin syndrome, cellulitis, etc. [Sampathkumar P, 2007; Lowy FD, 2003].

Nasal carriage of *S. aureus* has been identified as a risk factor for community-acquired as well as nosocomial infections [Cole AM et al, 2001]. The relatively high percentage (25-30%) of adults who are colonized by *S. aureus* in the nose have Staphylococci present that do not cause illness, and only about 2% of healthy people carry Methicillin-resistant *Staphylococcus aureus* (MRSA) in the nose with no symptoms [Darouiche RO, 2001]. MRSA also called “superbug”, is a type of *Staphylococcus aureus*, an important pathogen which are resistant to methicillin, penicillin, amoxicillin and oxacillin [Wenzel RP et al, 1991]. It was first reported in 1961 within a year of methicillin introduction [Lowy FD, 2003]. Since then, MRSA strains have spread among hospitals and disseminated worldwide. The National Nosocomial Infections Surveillance System (NNISS) reported an increase of MRSA in large U.S. hospitals from 34% in the 1980s to 50% in the late 1990s. In some hospitals, methicillin-resistant strains represented up to 80% of all *S. aureus* strains [Oliveira DC et al, 2002; Wenzel RP et al, 1991].

MRSA formerly appeared in patients in hospitals and other health facilities, especially among the elderly, the very sick, and those with an open wound such as a bedsore or catheter in the body and referred to as health care-associated MRSA (HA-MRSA) [Hiramatsu K et al, 2001]. It has also been found to cause illness in the community outside of hospitals and other health facilities which is known as community-associated MRSA (CA-MRSA). The U.S. Centers for Disease Control and Prevention (CDC) estimates that about 12% of MRSA infections are now community-associated, but this percentage can vary by community and patient population due to recent antibiotic use, sharing contaminated items, having active skin diseases or injuries, poor hygiene and living in crowded settings [Grundmann H et al, 2006].

MRSA strains easily colonize a host particularly in immunodeficient patients and can cause a variety of serious infections [Rikitomi N et al, 1994; Rubio M et al, 1999]. The transmission of MRSA is largely from people with active MRSA skin infections and spread always by direct physical contact except through the air and indirect contact by touching objects such as towels, sheets, wound dressings, clothes, workout areas, sports equipment contaminated by the infected skin of a person with MRSA [Grundmann H et al, 2006].

The prevalence of MRSA is increasing continuously from 2009-2011 by 54% [Rahman S et al, 2011]. In Nepal, prevalence of the MRSA is fluctuating and ranging from 11.7% to 54.9% [Khanal L and Jha B, 2010; Kumari N et al, 2008; Rajbandari R, 2002; Rijal KR et al, 2008]. The proposed risk factors associated with the increased prevalence of MRSA are indiscriminate use of antibiotics, prolonged hospitalization, intravenous drug abuse etc. The morbidity and the mortality of infectious diseases have increased proportionately with the acquisition of antibiotic resistant by MRSA, especially in regards to the strains that are completely resistant to antibiotics. Therefore, the present study was conducted to determine the prevalence of MRSA from upper respiratory tract infection and to find out its antibiotic susceptibility pattern.

**MATERIALS AND METHODS**

**Study Design**

The present research work was a descriptive cross-sectional study conducted at the Department of Microbiology, Clinical Laboratory Sciences of Janaki Medical College Teaching Hospital (JMCTH), Janakpur, Nepal from May to November 2015.

**Study Population**

This study comprised 378 clinically ill, both OPD and ward patients including both sexes of all age groups visiting Janaki Medical College Teaching Hospital.

**Sample Collection and Processing**

Nasal swab of OPD and ward patients was collected from nasal cavity in a clean, sterile, dry test tube. The samples were taken to the laboratory and processed as quickly as possible. Media were prepared as instructed by the manufacturer company (Hi-media). The swab were streaked onto plates of MacConkey agar (MA), Blood agar (BA) and Mannitol salt agar (MSA) and incubated aerobically at 37°C for 24 hours.

**Identification of the Isolates**

After incubation, visual growth on the inoculated plates was observed and colony morphology was noted. Identification of the isolates was done by using standard microbiological techniques as described in the Bergy's Manual which involved morphological appearance of the colonies, Gram's staining and biochemical properties.

**Identification of Gram Positive Isolates**

Gram positive organisms were tested by haemolysis pattern on blood agar, motility, catalase tests, oxidase...
Identification of Gram negative Isolates

The identification of various Gram negative isolates were done by using standard microbiological techniques described in Bergey's manual of Systematic Bacteriology (2nd edition). The isolates were identified on the basis of various biochemical tests such as catalase test, oxidase test, O/F test, MR/VP test, SIM test, citrate test, urease test, TSI test.

Antibiotic sensitivity test

Antibiotic sensitivity test for the isolated organism were done by using Kirby Bauer Disc Diffusion Method. Bacterial inoculums were prepared by suspending the freshly grown bacteria in 25 ml sterile Nutrient broth and compared with turbidity equivalent to 0.5 Mc Farland standard and was streaked on entire Muller-Hinton agar plate. Antibiotic discs were placed around the outer edge of the plate and incubated overnight at 37 ºC. Diameter of zone of inhibition was measured and zone diameter criterion was used to interpret the level of susceptibility to each antibiotic [CLSI 2013].

Screening of MRSA

After the antibiotic sensitivity testing of each of Staphylococcal isolates, the organisms were screened for MRSA. The MRSA strains were identified by testing with cefoxitin dics having zone of inhibition less than 21 mm.

Ethical Consideration

Ethical approval was taken from the Institutional Ethical Committee. A paper of information letter and consent form was given to patients before participating in the research. In case of illiterate participants, information was provided by reading the consent form in presence of witness. The information of patients was collected such as name, age and sex.

Statistical Analysis

The data were analyzed using SPSS 16.0 version statistical software and Microsoft excel's 2007. The p-value < 0.05 was considered statistically significant used to determine the association between patients with MRSA.

Quality Control

Laboratory equipment like refrigerator, incubator, autoclave and hot air oven were regularly monitored for their efficiency. The temperature of refrigerator and incubator was monitored everyday for their performance and immediately corrected if any deviation occurred. Reagents and media were regularly monitored for their manufacture, expiry date and proper storage. After preparation, they were properly labelled with preparation date. The quality of media prepared was checked by incubating one plate of each lot for sterility and using standard control strains for performance testing. During identification of organisms, for each test Staphylococcus aureus (ATCC 25923) was used as reference strains for culture and sensitivity testing. Strict aseptic conditions were maintained while carrying out all the procedures.

RESULTS

Pattern of Microbial Growth

Altogether 378 samples were collected from nasal cavity of OPD and ward patients. Of them, 116 (31%) showed positive growth and rest of them showed no growth (Figure 1). From positive growth, 102 (87.93%) were Gram positive and 14 (12.06%) were Gram negative isolates (Figure 2).

Figure 1: Distribution of bacterial growth

Figure 2: Distribution of Gram's staining bacterial isolates
Genderwise Distribution of *Staphylococcus* species

Of Gram positive, 98 were Staphylococci and rest was other. Out of total staphylococci, 71 were *S. aureus* and 27 were CoNS. Both *S. aureus* and CoNS were more frequently isolated from female with 53.5% and 70.4% respectively (Figure 3).

Antibiotic Sensitivity Pattern of *Staphylococcus* species

*S. aureus* were more sensitive towards gentamicin and vancomycin with 88.7% followed by cefotaxime, tetracycline and ciprofloxacin with 85.9%, 80.3% and 64.8% respectively and maximum resistance towards penicillin and ampicillin with 81.7% followed by 43.7% cotrimoxazole and 38% cefoxitin. The results are shown in Figure 4.

Pattern of Methicillin resistant *S. aureus* (MRSA)

Out of 71 isolates of *S. aureus*, 28% isolates were defined as MRSA. Cefoxitin disk (MR) disk diffusion method was used for the detection of hetero-methicillin resistant *S. aureus*. The results are shown in Figure 5.

Distribution of MRSA in different age group

The highest numbers of MRSA were found in the age group 21-40 years with 44.44%, followed by 1-20 years with 25.92%. The results are shown in Table 1.

<table>
<thead>
<tr>
<th>Age groups (yrs)</th>
<th>Types of organisms</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em> No. (%)</td>
<td>MRSA No. (%)</td>
</tr>
<tr>
<td>1-20</td>
<td>25 (35.21)</td>
<td>7 (25.92)</td>
</tr>
<tr>
<td>21-40</td>
<td>25 (35.21)</td>
<td>12 (44.44)</td>
</tr>
<tr>
<td>41-60</td>
<td>10 (14.080)</td>
<td>4 (14.81)</td>
</tr>
<tr>
<td>61-80</td>
<td>9 (12.67)</td>
<td>3 (11.11)</td>
</tr>
<tr>
<td>&gt;80</td>
<td>2 (2.81)</td>
<td>1 (3.70)</td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>27</td>
</tr>
</tbody>
</table>

Distribution of MRSA among gender

The highest number of MRSA was isolated from female patients with 51.85% which was found to be statistically insignificant (p=0.128). The results are shown in Table 2.
Distribution of MRSA among OPD and Ward patients

The highest prevalence of MRSA was found in ward with 70.37% than OPD (29.62%) and was statistically significant (p=0.001). The results are shown in Table 3.

Table 3: Distribution of MRSA among OPDs and Ward patients

<table>
<thead>
<tr>
<th>OPD/Wards</th>
<th>S. aureus No.</th>
<th>MRSA No.</th>
<th>Total</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPD</td>
<td>22</td>
<td>8</td>
<td>30</td>
<td>p=0.001</td>
</tr>
<tr>
<td>Ward</td>
<td>49</td>
<td>19</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>27</td>
<td>98</td>
<td></td>
</tr>
</tbody>
</table>

Antibiotic Susceptibility Pattern of MRSA Isolates

Of total MRSA isolates, most of the MRSA isolates showed equal sensitive pattern toward vancomycin and tetracycline with 85.19% followed by gentamicin (74.07%), cotrimoxazole and cefotaxime with 66.67%. The results are shown in Figure 6 and 7.

DISCUSSION

Surveillance on the antimicrobial susceptibility patterns of S. aureus is of chief importance in understanding new and emerging resistance trends and in the management of both hospital and community-acquired infections. This is a great concern due to the high rates of resistance to antimicrobials used in the treatment of infections caused by pathogens particularly in developing countries. In recent years, the extensive and inappropriate use of antimicrobial agents has continually resulted in the development of antibiotic resistance which has become a major public problem worldwide as infection caused by MDR strains often leads to death [Yadav K and Prakash S, 2016].

S. aureus has always been a faltering block for antimicrobial chemotherapy and the introduction of new classes of antimicrobial agents is usually followed by the emergence of resistant forms of this pathogen [Hiramatsu K et al, 2001; Kim HB et al, 2004]. The relationship between antimicrobial consumption and MRSA is well established and was found to be dose dependent in defined healthcare settings [Monnet DL, 1998; Tacconelli E et al, 2008]. In hospitals, antimicrobial resistance leads to increased healthcare costs primordially due to a higher morbidity and mortality from infectious diseases which amplified length of stay [Cosgrove SE et al, 2003].

This study revealed that a total of 98 staphylococci isolated from upper respiratory tract, 71 were S. aureus, 27 were CoNS and rest were others. Similar findings were also obtained in the study conducted by Khadri and Alzoharly, 2010 and Shrestha C, 2012 which is in accord with our study. The present study also found both
S. aureus and CoNS were more frequently isolated from female with 53.5% and 70.4% respectively which could be due to more flow of female patients in the hospital. This study showed S. aureus were more sensitive towards gentamicin and vancomycin with 88.7% followed by cefotaxime, tetracycline and ciprofloxacin with 85.9%, 80.3% and 64.8% respectively. Seybold U et al, 2006 reported the degree of susceptibility of S. aureus to vancomycin and gentamycin to be 91.7% in review of similar work. Similarly, Rijal KR et al, 2008 reported 96.9% susceptibility of S. aureus isolates to vancomycin. The isolated S. aureus were highly resistant to commonly prescribed antibiotics in the present study. The maximum resistance towards penicillin and ampicillin with 81.7% followed by 43.7% cotrimoxazole and 38% cefotixin. The highest resistant pattern was shown for penicillin and ampicillin with 81% resistance. Similar types of resistant pattern were shown in various previous studies. Study conducted by Shrestha C, 2012 showed 94.85% resistant towards penicillin. Many factors may have contributed to such level of resistance, including misuse of antibiotics by health professionals, unskilled practitioners and lay persons. In Nepal, it is a common practice that antibiotics can be purchased without prescription, poor hospital hygienic conditions, inadequate surveillance which leads to misuse of antibiotics by the public contributing to the emergence and spread of antimicrobial resistance [CDC, 2006].

The present study revealed 28% MRSA was identified from total Staphylococci isolated from upper respiratory tract. Agmy G et al, 2013 reported 23% MRSA strains from URTI which reflects increasing trend of MRSA and is almost similar to this study. But, the result of the present study differ with the findings of study conducted by Rajaduraiapandi K et al, 2006 and Sanjana RK et al, 2010 with 37.9% and 39.9% respectively which may be due to increased flow of patients. The highest number of prevalence of MRSA were found in the age group 21-40 years with 44.44%, followed by 1-20 years with 25.92%. A similar finding was also obtained by Kahasay A et al, 2014 which is similar to this study. This may be due to high active age group and is more exposed to injuries. This study showed the highest number of MRSA was isolated from female patients slightly more than male patients which was found to be statistically insignificant (p=0.128). Among total MRSA isolates, 70.37% was isolated from ward and 29.63% was from OPD and found to be statistically significant (p=0.001). This difference could be due to various hospital associated risk factors such as prolonged hospital stay, antibiotic treatment, underlying immune-compromised condition, instrumentation and other invasive devices which predispose patients to MRSA acquisition. The similar study conducted by Thapa KB, 2011 and Sanjana RK et al, 2010 also showed the higher percentage of MRSA isolated from ward rather than OPD.

Of total MRSA isolates, most of the MRSA isolates showed equal sensitive pattern toward vancomycin and tetracycline with 85.19% followed by gentamicin (74.07%), and also equal sensitive pattern cotrimoxazole and cefotaxime with 66.67% whereas 92.60% of MRSA showed resistant towards ampicillin and penicillin. Similarly, MRSA was found to be more resistant towards ciprofloxacin with 59.45%. The irrational use of antibiotics without surveillance might contribute to emergence of MDR strains. The lack of legislation for practical use of antibiotics leads to indiscrimination, irrational use of antibiotics without prescription and termination of antibiotics before full course is one of the major cause of emergence of MDR organisms in the country [Thapa KB, 2011].

CONCLUSION

Nepal is one of the developing countries facing a high prevalence of MRSA in all age groups which is life threatening. The present study concluded MRSA infection is still one of the most threatening infections in the hospital of Nepal as such infections are difficult to treat. It was further concluded that prevalence of MRSA was found to be in an increasing trend compared to previous studies. Moreover, abuse of antibiotics has led to the emergence of Multi Drug Resistant (MDR) bacteria which are difficult to control as these bacteria are resistant to most of the antibiotics. The rise of MRSA and MDR cases is a major problem in today's health context which encountered among diseased persons. There is need of regular interval time surveillance in order to monitor resistance pattern. Also, self medication, inadequate or excessive medication should be discouraged. Hand washing, use of gloves, gown and masks, isolation of MRSA from patients, regular decolonization of the hospital environment and use of suitable disinfectants in clinical use are likely to reduce the spread of MRSA.

ACKNOWLEDGEMENT

Authors are profusely thankful to hospital management committee and Department of Clinical Pathology, Janaki Medical College Teaching Hospital (JMCTH), Janakpurdham, Nepal for creating a research environment and providing their steady source of assistance during this research.

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