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Nasal Colonization of Methicillin-Resistant *Staphylococcus aureus* among Healthcare Workers at a Tertiary Care Hospital in Kathmandu, Nepal

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***Staphylococcus aureus* is a common bacterium with an increasing level of antibiotic resistance. The main objective is to assess the nasal carriage rate of methicillin-resistant *S. aureus* among healthcare workers. A total of 50 nasal swabs were collected from healthcare workers at the tertiary care hospital of Kathmandu Valley. To identify *S. aureus*, 50 nasal swabs were treated with standard bacteriological procedures. A modified Kirby-Bauer disc diffusion test was performed to test antibiotic susceptibility. Out of 50 samples, 28% were determined to be carriers of *S. aureus,* with females having a proportion of 32.25% and males, 31.57%. The nasal carriage rates were highest in the 40 to 50 age group (23.81%), and rates were highest in the nurse (42.85%). Co-trimoxazole (81.25%) was the most effective antibiotic against *S. aureus* strains, followed by chloramphenicol (62.5%), ciprofloxacin (56.25%), erythromycin (31.25%), and amoxicillin (18.75%). Among the 28 *S. aureus* isolates, 12.5% were confirmed as MRSA, with a higher prevalence observed in males (16.66%) than in females (10%). The MRSA rate was highest in the 40 to 50 age group (16.66%), and the rate was highest in the nursing home (22.225%). Among the 16 isolates, 10 isolates for Methicillin Sensitive *S. aureus* exhibit multi-drug resistance (MDR), whereas all MRSA isolates were considered to be MDR. The study concluded that MRSA prevalence is still increasing. Enhancing hospital hygiene standards for both staff and visitors will help in preventing the spread of *S. aureus* and MRSA.**

Keywords: Antibiotic resistance, Health care workers, Multi-drug resistance, MRSA, *Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus is a normal flora found in the human skin, nasopharynx, perineal region, and anterior nares. It can colonize a variety of mucosal or epithelial surfaces. Endogenous strains from patients can spread to normally sterile sites through traumatic introduction, such as surgical wounds or micro abrasions, from person to person through fomites, air, or unwashed hands of healthcare workers, particularly in nosocomial settings. *S. aureus* is a leading cause of hospital and community-acquired infections globally, leading to significant morbidity and mortality (1). Resistant to more than two antibiotics of completely different structural classes is known as multi-drug resistance (2). Compared to illnesses obtained in the community, drug resistance is more common in infections acquired in hospitals. Antibiotics commonly used in hospitals target these bacteria, contributing to this issue. The hospital strains are characterized by their simultaneous development of antibiotic resistance. Isolates that are resistant to many drugs are significantly more likely to cause issues when treating patients with infectious illnesses. MDR in most pathogens has led to treatment failures and increased costs associated with keeping these patients under control, making early and reliable identification of these infections' imperative.

Globally, the MRSA rate has been rising, particularly in hospital-acquired cases (3). Several mechanisms lead to methicillin resistance in staphylococci, including the development of the mecA gene that encodes for a penicillin-binding protein with a poor affinity for beta-lactams, deactivation by beta-lactamase enzymes, and decreased penicillin-binding abilities in penicillin-binding proteins. Most resistance to several beta-lactams, including methicillin, is explained by the latter mechanism (4). MRSAs are those that have the PBP-encoding mecA gene. The gene known as mecA is present on a genomic component called SCC mec, which leads to resistance to antibiotics (5). Since the 1960s, these isolates have been identified as a reason for illness linked to healthcare, and they are resistant to all β-lactam drugs now on the market, including β-lactamase stabilized penicillin (6). Healthcare facilities struggle to conduct accurate antibiotic susceptibility testing because of a shortage of basic infrastructure and staff with knowledge. As a result, data on the prevalence of MRSA throughout all of Nepal is unavailable. The MRSA rate of Nepal covers from 11.7% to 54.9%. The rise of MDR *S. aureus* in hospitals has also been documented in numerous studies (7). The results unambiguously show what needs to be done in hospital settings to minimize nosocomial infections by lowering MRSA and MDR strains.

The globe is becoming increasingly concerned about antibiotic resistance when it comes to treating bacterial illnesses. MRSA is a global health concern and a public burden, particularly in developing nations such as Nepal, due to inadequate health-care standards and illogical antibiotic use. This study will provide the current MRSA

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situation as well as the pattern of antibiotic sensitivity. This is extremely helpful for planning the future and developing regulations in hospitals and healthcare facilities to stop the spread of infection that can be fatal. Similarly, sampling from the hospital environment can be valuable in determining the source of infection and avoiding the spread of dangerous pathogens.

MATERIALS AND METHODS

Ethical approval and consent to participate: The Approval was received from D.A.V. College, Department of Microbiology, and individual consent was obtained from each healthcare worker.

Collection of samples: Nasal swabs were collected from doctors, nurses, lab technicians, pharmacists, and paramedical officers. Samples were collected with a sterile cotton swab that had been dipped in physiological saline. It was inserted 1-2 centimeters into the first nares and gently rotated 2-3 times in both clockwise and anticlockwise directions for 3-5 seconds. The process was then repeated when it was moved to the second nares. After the sample was taken, the swab was immediately placed in the sterile specimen tube, securely capped, properly labelled with a sample code number, and transported to the laboratory within one hour of collection for further processing (8).

Sample processing: Standard methods were followed in the processing of every sample chosen for investigation. The samples were inoculated into MSA and incubated at 37˚C for 24 hours.

Identification of *S. aureus*: Mannitol salt agar (Himedia) was used to create the primary inoculum. The isolates that were yellow colonies on MSA and gram-positive cocci were subcultured on nutrient agar (NA) and incubated for 24 hours at 37ºC. The golden yellow colony of staphylococci was used to identify them. To confirm *S. aureus*, isolated colonies were tested by Gram reaction, catalase test, oxidase test, oxidation-fermentation test, and coagulase test (9).

Antibiotic susceptibility testing: The antibiotic disc susceptibility test was performed using Kirby Kirby-Bauer disc diffusion technique explained in CLSI 2014. Nutrient broth was used to transfer four to five colonies, and the suspension was examined against 0.5 MCFarland. Antibiotics like amoxicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), co-trimoxazole (25 µg), erythromycin (10 µg), and cefoxitin (30 µg) were used. MRSA strains were identified by testing with cefoxitin (30 µg); strains that have an inhibitory zone diameter ≤ 21 mm were considered as methicillin resistant, while strains having a diameter > 22 mm were considered as methicillin susceptible (6).

Data analysis: Data were entered in MS Excel 2007 (12.0.6787.5000), and the interpreted data were presented in the form of diagrams, graphs, and tables to allow for a visual appreciation of various analytical features.

RESULTS

Total isolates: Out of 50 nasal swabs, 16 (32%) swabs were found to be carriers of *S. aureus*, while 34 (68%) swabs were not.

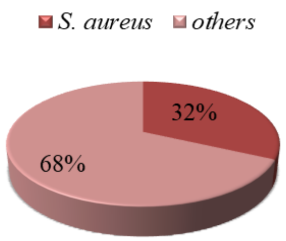

Figure 1: Total isolates of *S. aureus* from nasal swab.

Gender wise nasal carriage of *S. aureus:* In this study of 50 nasal swabs, 19 from males and 31 from females, *S. aureus* was isolated in 6 (31.57%) of the male samples and 10 (32.25%) of the female samples (Table 1).

Table 1: *S. aureus* isolated from male and female (n=50).

Gender	Total sample	No. of positive isolates	Percentage of positive isolates
Male	19	6	31.57
Female	31	10	32.25
Total	50	16	32

Age-wise nasal carriage by *S. aureus*: Age group was obtained during sample collection and was classified into predefined age categories. Among the samples taken in different age groups 40 to 50 age group had the highest nasal carrier of *S. aureus* (23.81%), which was followed by 31.81% for people aged 30 to 40, 25% % for those aged 20-30, and 14.28% for those aged >50 (Table 2).

Table 2: Age-wise distribution of *S. aureus* in the study group.

Age group (Years)	Total sample	No. of positive isolates	Percentage of positive isolates
20-30	8	2	25
30-40	22	7	31.81
40-50	13	6	46.15
>50	7	1	14.28
Total	50	16	32

Profession-wise nasal carrier of *S. aureus*: During the brief interview conducted at the time of sample collection, the occupation of each participant was recorded. The hospital staff who are involved in the healthcare facility were further divided into doctors, nurses, lab technicians, pharmacists, and paramedical officers. Nasal carriage rate of *S. aureus* was found to be highest among nurses (42.85%), followed by lab technicians (33.33%) and doctors (28.57%) (Table 3).

Table 3: Profession-wise nasal carrier of *S. aureus*.

Profession	Total sample	No. of positive isolates	Percentage of positive isolates
Doctor	14	4	28.57
Nurse	21	9	42.85
Lab technician	9	3	33.33
Pharmacists	4	0	0
Paramedical officers	2	0	0
Total	50	16	32

Antibiotic susceptibility pattern of *S. aureus* isolates: Kirby Kirby-Bauer disc diffusion method was used to examine each strain that was isolated from a nasal swab of healthcare workers. The table showed that co-trimoxazole (81.25%) was the drug that was the most sensitive to *S. aureus* strains, followed by chloramphenicol (62.5%), ciprofloxacin (56.25%), erythromycin (31.25%), and amoxicillin (18.75%) (Table 4).

Table 4: Antibiotic susceptibility pattern of isolates (n=16).

Antibiotics	Number and percentage of isolates	
	Sensitive	Resistance
Amoxicillin	3(18.75%)	13(81.25%)
Chloramphenicol	10(62.5%)	6(37.5%)
Co-trimoxazole	13(81.25%)	3(18.75%)
Ciprofloxacin	9(56.25%)	7(43.75%)
Erythromycin	5(31.25%)	11(68.75%)
Cefoxitin	14(87.5%)	2(12.5%)

Methicillin-resistant *S. aureus* (MRSA): MSSA and MRSA were the two categories into which the isolated strains were divided. It was found that 87.5% isolates were MSSA and 12.5% were cefoxitin (MRSA) resistant (Figure 2).

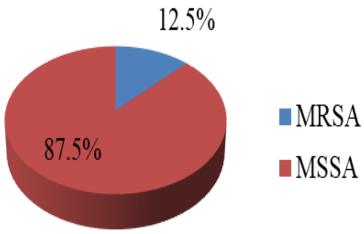

Figure 2: Distribution of MRSA and MSSA from study population.

Multi-drug resistance (MDR): The isolated strains of *S. aureus* were divided into two groups multi-drug resistance -MRSA and non multi drug resistance -MRSA. Multidrug resistance was identified based on antibiotic resistance patterns. Multidrug resistance was identified based on antibiotic resistance patterns. Out of 16 S. aureus isolates, 10 isolates of MSSA were multidrug resistant. All MRSA isolates were MDR (Figure 3).

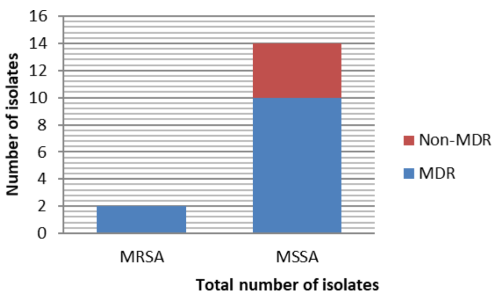

Figure 3: Comparative chart showing MDR and non-MDR in MRSA and MSSA.

MRSA distribution among male and female: Out of the total MRSA isolates from health care workers, 16.66% were found to be male and 10% to be female.

MRSA distribution among different age: MRSA were most common in the age group 40-50 (16.66%), followed by the age group 30-40.

MRSA distribution in relation with profession: Among 9 *S. aureus* isolates from nurse, 22.22% were MRSA. No MRSA was found from doctors, paramedical officers, lab technicians and pharmacists.

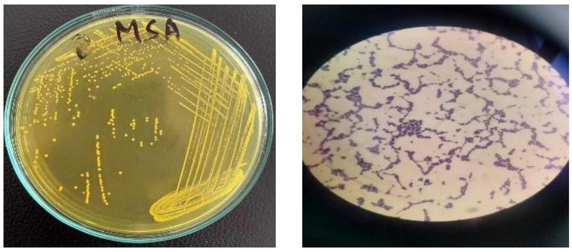

Figure 4: Yellow colony on Mannitol salt agar and Gram staining *of S. aureus*.

DISCUSSION

Many healthy individuals carry *S. aureus* in their nasal passages and other body surfaces, leading to rapid spread of Staphylococcal infections, particularly in hospitals. Healthy hospital staff members may have nosocomial strains in their nares, which they might then transmit to the population and worsen existing conditions. Healthcare workers who operate as a bridge between the hospital and the community may also act as transmitters of *S. aureus* and MRSA. Therefore, studying *S. aureus* and MRSA is crucial for healthcare professionals to develop a clear understanding of its presence.

In the present study, overall, 16 (32%) participants were identified as nasal carriers of *S. aureus.* The result is greater than the finding of Khanal *et al*., 2005 (8). Additionally, previous studies have reported nasal carriage rates ranging from 18% to 50% worldwide across different populations, and the pathogenesis and epidemiology of infection seem to be significantly influenced by *S. aureus* carriage in the nose. This could be due to the research location's unique environmental, climatic, and geographical conditions (10).

Among 16 *S. aureus* isolates, 31.57% were male, and 32.25% were female, which is more than the findings of Shah, 2002 (7). According to Shah, 2002 (7), nasal colonization of *S. aureus* in males was 19% and in females 21.2% in the hospital of Kathmandu valley. In this study, females exhibited higher nasal carriage of *S. aureus* than males, showing that sex might be a factor for colonization and that female activity contributes to colonization. The finding is in opposition to Onanuga *et al*., 2005 (11), who revealed that colonization was not associated with sex.

The 40-50 age group had the highest nasal carriage rate of *S. aureus*. A similar study also reported that individuals aged 36 to 45 years had the highest percentage of nasal carriers (33.3%), while those aged 46 years and above had the lowest rate (4.8%) (12).

In the present study, the nasal carriage rate was highest among nurses. A similar study conducted in a Nepalese hospital also found an elevated prevalence of *S. aureus* nasal colonization among nurses (13). Healthcare staff are repeatedly exposed to colonized patients, and their carriage rate may increase during extended work hours. This finding underscores the need for strengthened infection-control practices targeting health care workers who are in continuous interaction with vulnerable patient groups.

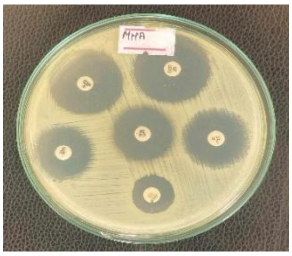

Figure 5: Antibiotic susceptibility test.

In the present study, co-trimoxazole showed the highest sensitivity to *S. aureus* strains, while amoxicillin exhibited the highest resistance. Resistance among *S. aureus* strains has increased due to inadequate hospital cleaning protocols, unhygienic practices, and a lack of antimicrobial agents monitoring on hospital premises, as reported by Ahmad *et al.*, 1998 (14).

The indiscriminate use of various antibiotics, particularly in developing nations, extended hospital stays, overuse of intravenous drugs, and MRSA transmission through the nose are thought to be some of the risk factors linked to a high incidence of MRSA infections. The current study revealed that 12.5% were MRSA. The MRSA rate was higher than that reported by Shakya *et al*., 2010 (15), who observed that 10% of nasal carriers were MRSA. But the MRSA carrier rate reported in the present study was lower than the report of Chen *et al*., 2005 (16), where 13.6% were MRSA.

Significant differences were seen in the sensitivity pattern of both *S. aureus* groups to antibiotics. Numerous investigations have reported an increasing prevalence of MDR *S. aureus* in medical facilities (7). These results unambiguously show what needs to be done in hospital settings to minimize nosocomial infections by lowering MRSA and MDR strains.

It has been found that 16.66% male and 10% females was carrier of MRSA. A similar outcome was observed in Khanal *et al*., 2015 investigation. They found higher carriage of MRSA in males (19.5%) than in females (11.5%) (8).

MRSA was not detected in the 20 to 30 and older age group. This may be the outcome of the small number of samples that were taken from the 20 to 30 and the older than 50 age groups.

Among 9 *S. aureus* isolates from nurses, 22.22% were MRSA. The outcome is comparable to the research where the MRSA carriage was high, particularly among the nurses (21.22%) (12). It's possible that the greater MRSA rate among nurses stems from their ignorance of infection control procedures and lack of experience in managing patients with MRSA infections. MRSA nasal carriage in healthcare workers increases the likelihood of transmitting the organism to patients during routine care.

CONCLUSIONS

The study determined that MRSA prevalence is still increasing. The presence of *S. aureus* and MRSA in healthcare workers (HCWs), especially in nurses, makes it necessary to regulate the frequency of their contact with susceptible patients and to implement stringent infection control measures in order to prevent nosocomial infections. The findings emphasize the importance of hospitals adhering to strict infection control guidelines. To prevent the spread of *S. aureus*, regular screening of hospital staff is recommended. An increased proportion of MDR strains highlights the need to prevent antibiotic misuse. The study emphasizes the need to reduce nasal carriage to minimize the spread of MDR strains.

CONFLICTS OF INTEREST

The authors have declared that no competing interests exist.

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