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Antimicrobial Resistance Pattern of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Strains Isolated from Clinical Specimens in Kathmandu, Nepal

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Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) refers to a group of Gram-positive bacteria that are genetically distinct from other strains of *Staphylococcus aureus* and is responsible for several difficult-to-treat infections in humans. It constitutes the most severe contemporary challenges to the treatment of hospital-acquired infections.

Objective: This study was conducted to determine the prevalence of MRSA among clinical isolates of Methicillin-resistant *Staphylococcus aureus* bacterium and determine the antimicrobial susceptibility pattern.

Methods: A cross-sectional study was carried out from March to September 2018 at Everest Hospital, Kathmandu, Nepal, in which 526 samples isolated from various clinical specimens. Methicillin-resistant *Staphylococcus aureus* (MRSA) identified by using the Cefoxitin (30 µg) disc diffusion method followed by the Clinical and Laboratory Standards Institute (CLSI 2015) guidelines.

Results: Most of the MRSA was isolated from pus, which collected from OPD patients. Antibiotic sensitivity pattern showed that all Methicillin-resistant *Staphylococcus aureus* isolates were sensitive to vancomycin teicoplanin, linezolid, and none of the isolates were sensitive to antibiotic penicillin. At the end of the final observations, 33.8% of clinical samples were positive to *S. aureus* growth.

Conclusion: In this study, the prevalence of MRSA isolates was less as compared to other studies routine screening of MRSA by cefoxitin disc and determining the complete antimicrobial susceptibility profile of these isolates to treat these infections effectively.

Keywords: Methicillin-resistant *Staphylococcus aureus* (MRSA); Cefoxitin; E-test; Clinical and bacteria

Introduction

Staphylococcus aureus, a member of the family *Micrococcaceae*, is a Gram-positive coccus whose cells tends to occur either singly or if dividing cells do not separate, form pairs, tetrads, and distinctive irregular "grape-like" structures [1]. Humans are commonly colonized by *S. aureus* on external skin surfaces and the upper respiratory tract, particularly the nasal passages. Healthy individuals are usually unaware of *staphylococcal carriage*, but they may suffer from minor skin infections such as boils and abscesses [2]. However, *S. aureus* is an opportunist pathogen, and given the right circumstances can cause more severe infections. Burns and surgical wound infections are commonly invaded by *S. aureus*, where the production of toxins gives rise to toxic shock syndrome leading to fever, sickness, and in some cases, death [3]. Infections caused by *S. aureus* include pneumonia, (inflammation of lungs), mastitis (infection of the mammary glands), infections of the skin (impetigo, cellulitis, and *staphylococcal* scalded skin syndrome), osteomyelitis (infection of the bone), endocarditis (infection of the endothelial lining of the heart and valves) and bacteremia (bacteria present in the blood) [4]. *S. aureus* can also cause food poisoning, the result of enterotoxin production. *Staphylococcus aureus* often referred to as "staph", is a common type of bacterium that is found in about 25 to 30 percent of healthy people, primarily on the skin or in the nose [5]. In the past few decades, a more dangerous form of staph has emerged, known as methicillin-resistant *Staphylococcus aureus*, and is usually referred to by the acronym MRSA.

The first type, healthcare-acquired MRSA, has been recognized since the 1960s while second type of MRSA which appeared in the 1990s and is known as community-acquired MRSA. MRSA [6] occurs outside of hospital settings and usually manifests itself as a skin infection in an otherwise healthy individual. MRSA can develop into a more serious, life-threatening illness and tends to occur under conditions where

people are in prolonged physical proximity, such as in childcare and long-term care facilities, and in soldiers, prisoners, athletes involved in skin-to-skin contact sports such as wrestling, and in individuals sharing personal items such as towels [7]. MRSA usually enters the body through a cut or scrape. The first sign of infection is commonly described as resembling a spider bite with a spot on the skin that is red, swollen, and painful. The site may produce pus. Infrequently, MRSA infection can progress to a more severe disease, such as bloodstream infection. The best defense against MRSA is to maintain good hygiene, including frequent and thorough hand washing, and to avoid the sharing of personal care items [8]. According to a report by the Centers for Disease Control and Prevention (CDC) in the United States in 2005, more 94,000 people developed life-threatening infections caused by MRSA and nearly 19,000 people died during hospital stays related to these MRSA infections [9]. Many MRSA cases, 85 percent, were associated with healthcare facilities, while approximately 14 percent occurred in individuals with no known exposure to healthcare. The incidence of disease caused by MRSA bacteria is increasing worldwide. MRSA in Nepal [10] has reported being 15-69% from different areas in the country, and various laboratories have reported the emergence of multidrug-resistant organisms such as extended-spectrum beta-lactamase-producing organisms (ESBL), vancomycin-resistant enterococcus (VRE), penicillin-resistant *Streptococcus pneumoniae* [11]. Thus, this research conducted to determine the antimicrobial resistance patterns and plasmid profiles of MRSA isolated from clinical samples at Everest Hospital, Kathmandu, Nepal.

Materials and Methods

Collection of samples

This study was done from March to September 2018 at Everest Hospital, Nepal. With the consent of clinic followed by Chief Medical Officer (CMO), the samples were collected from a total of 526 patients for the routine culture of MRSA in the Clinical Laboratory, Department of Microbiology, IOM, Tribhuvan University, Kathmandu, Nepal [12]. Consent from the patient also taken individually, followed by the age, sex, date, and type of patient (inpatient and outpatient) with antibiotic sensitivity was recorded manually through a request form provided by the hospital. The complete process of sample collection and analysis procedures has explained in **Figure 1**.

Culture and identification of MRSA

Staphylococcus aureus isolates (**Figure 2**) were subjected to Cefoxitin disc diffusion testing using a 30 µg cefoxitin disc. The results interpreted according to guidelines given by the Clinical and Laboratory Standards Institute [13]. An inhibition zone diameter of ≤ 21 mm reported as methicillin-resistant and ≥ 22 mm reported as methicillin-sensitive.

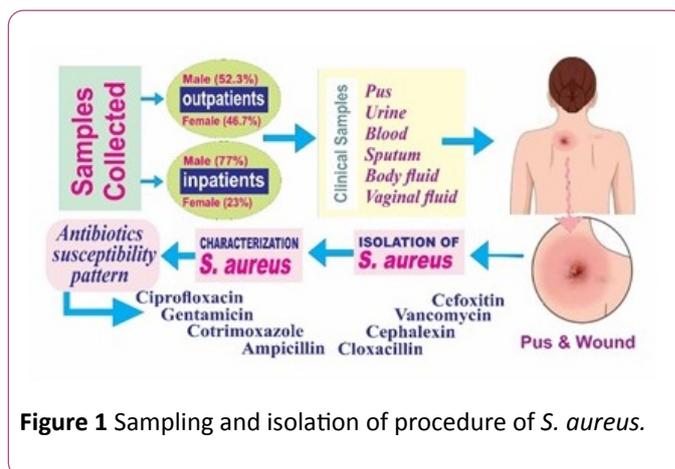


Figure 1 Sampling and isolation of procedure of *S. aureus*.

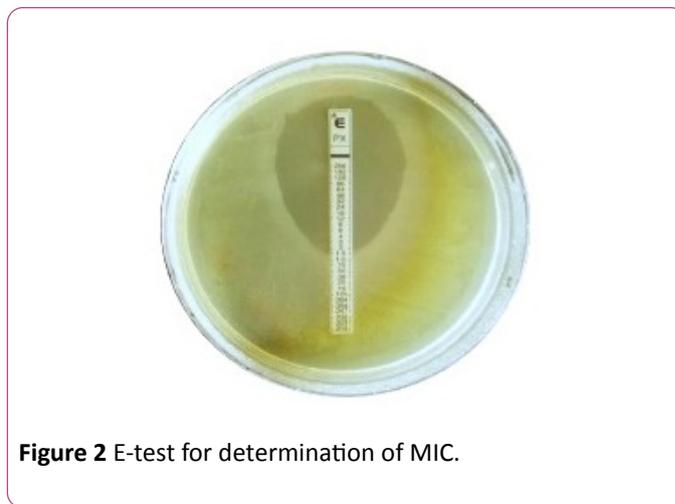


Figure 2 E-test for determination of MIC.

Antimicrobial susceptibility testing

All MRSA isolates were determined using disks of 8 antimicrobials on Mueller-Hinton agar media by modified Kirby Bauer's disc diffusion method as described in the guidelines of CLSI (2015).

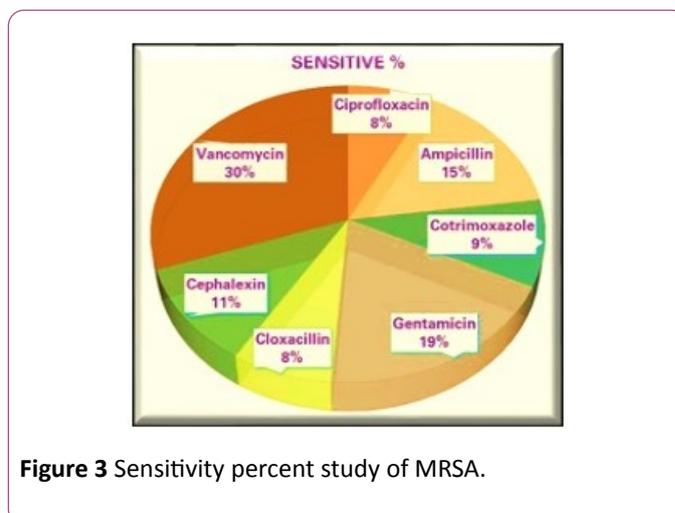


Figure 3 Sensitivity percent study of MRSA.

Since the Kirby Bauer disc diffusion method is not recommended for susceptibility testing of *Staphylococcus aureus* to the vancomycin, determination of minimum inhibitory concentration (MIC) was done either by E-test

(Figure 3) agar dilution method. When pure culture obtained, a loopful of bacteria was taken from a colony and was transferred to the tube containing 5 ml normal saline and mixed gently to obtain a homogenous suspension [14].

The turbidity of the suspension was then adjusted to a density of McFarland 0.5 to standardize inoculum size. A sterilized cotton swab dipped into the suspension, and then the excess was removed by gentle rotation of the swab against the surface of the tube [15]. The swab was then used to distribute the bacteria evenly over the entire surface of Muller Hinton agar. The inoculated plates left for 3-5 minutes at room temperature, and with the aid of sterile forceps, the following concentration of antibiotics discs put on the surface of Muller Hinton Agar 50 µg glucose 6-phosphate and incubated for 16-18 hrs at 37°C. Zone of inhibition after incubation was observed, and the diameter of inhibitory zones measured in millimeters (mm). Isolates having intermediate susceptibility were added in the total resistant isolates to each antibiotic tested. In this study, we used vancomycin E-strips (bio-Merieux) to determine the MIC of vancomycin. MIC ≤2 µg/ml interpreted as sensitive according to CLSI 2015 guidelines.

Statistical analysis

Data analysis has carried out with the Statistical Package for Social Sciences (SPSS) version 23.0 (SPSS, Chicago, IL, USA) using the Chi (χ^2) tool. The differences in data were considered statistically significant at $p < 0.05$.

Results

In this experiment, a total of 526 non-repeated clinical samples subjected to culture from both inpatient and outpatient over 6 months, and after the analysis, results were generated using standard statistical data.

Distribution of *S. aureus* from other organisms

Out of 526 clinical samples, a growth of different organisms was seen in 177 (33.6%) samples *S. aureus* was isolated from 60 (33.8%) different clinical samples (**Figure 4**).

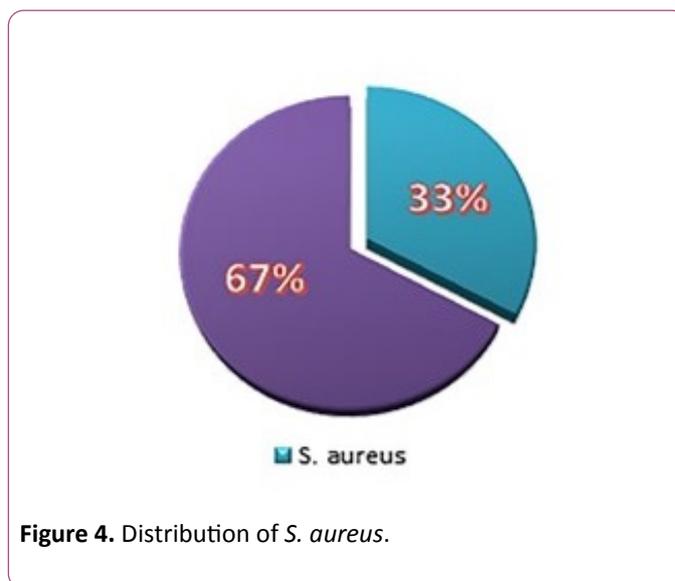


Figure 4. Distribution of *S. aureus*.

Distribution of total inpatients and outpatients among gender

Of the total 526 patients, 422 (80.2%) has obtained from Outpatient, and 104 (19.8%) samples obtained from Inpatients (**Table 1**). Of the total 305 male patients, 26.2% of samples were from inpatient and 73.7% from Outpatient. Similarly, of 221 female patients, 10.8% from inpatient, and 89.1% from outpatient (**Table 1**).

Table 1 Distribution of total patients among the gender.

Gender	Inpatient (%)	Outpatient (%)	Total
Male	80(26.2)	225(73.7)	305
Female	24(10.8)	197(89.1)	221
Total	104	422	526

Age and gender-wise distribution of total patients

The maximum number of samples (**Table 2**) was from male outpatients 225 (52.3%), followed by female outpatients 197 (46.7%). Male inpatients were 80 (77%), and female inpatients were 24 (23%). Maximum number of samples, i.e., 149 (75.6%) were from female outpatients of age 21-30 followed by male outpatients of same age group, i.e., 146(64.9%).

Table 2 Maximum number of samples collected from inpatients and outpatients.

Age group	Inpatients		Outpatients		Total (%)
	Male N (%)	Female N (%)	Male N (%)	Female N (%)	
1-10	0(0)	1(4.2)	1(0.4)	0(0)	2(0.4)
11-20	8(10)	6(25)	20(8.8)	21(10.6)	55(10.4)
21-30	26(32.5)	12(50)	146(64.9)	149(75.6)	333(63.3)

31-40	34(42.5)	1(4.2)	43(19.1)	16(8.1)	94(17.8)
41-50	0(0)	1(4.2)	8(3.5)	5(2.5)	14(2.7)
51-60	0(0)	3(12.5)	3(1.3)	5(2.5)	11(2.1)
61-70	11(13.7)	0(0)	3(1.3)	1(0.5)	15(2.8)
>70	1(1.2)	0(0)	1(0.4)	0(0)	2(0.4)
Total	80	24	225	197	526

Distribution of total isolates in clinical specimens

Among the investigated total of 526 clinical specimens, 176 (33.7%) specimens showed growth of organisms. Of the 177

positive growths, *S. aureus* was isolated from 60(33.8%) clinical specimens. *S. aureus* 89.7% were isolated from pus. In total, 107 (60.8%) growth in urine, 52(48.6%) Escherichia coli found (Table 3).

Table 3 Distribution of total isolates in clinical specimens.

Organism	Type of Sample				
	Urine N(%)	Pus swab N(%)	Blood N(%)	Sputum N(%)	Others N(%)
<i>S. aureus</i>	2(1.9)	52(89.7)	1(100)	3(33.3)	2(50)
<i>E. coli</i>	52(48.6)	0(0)	0	0	0
<i>Klebsilla spp</i>	35(32.7)	2(3.4)	0	0	0
<i>Proteus spp</i>	3(2.8)	0(0)	0	0	0
CONS	0(0)	2(3.4)	0	0	0
<i>Ps. Aeruginosa</i>	15(14)	2(3.4)	0	6(66.6)	2(50)
Total	107(60.8)	58(32.9)	1(0.5)	9(5.0)	4(2.2)

Isolation of *S. aureus* from different clinical samples

Maximum *S. aureus* 52(86.6%) isolates have obtained from pus swab followed by sputum 3(5%). Many specimens

comprised of urine, but only 2(3.3%) *S. aureus* was obtained from the specimen, which is like body fluids. Whereas 1(1.6%) from blood, there are no isolates from vaginal fluids (Table 4).

Table 4 Isolation of *S. aureus* from clinical samples.

Clinical Samples	Total samples (%)	<i>S. aureus</i> isolated (%)
Pus	128(24.3)	52(86.6)
Urine	246(46.7)	2(3.3)
Blood	70(13.3)	1(1.6)
Sputum	44(8.36)	3(5)
Body fluid	28(5.32)	2(3.3)
Vagina fluid	10(1.9)	0(0)

Antibiotics susceptibility pattern of *S. aureus*

All *S. aureus* isolates were found to be sensitive to Vancomycin and were least sensitive to Ciprofloxacin (41.6%). Isolates (Table 5) showed 85% sensitivity to Cefoxitin, whereas showed 73.3% sensitivity towards Gentamicin, Cloxacillin, and Cephalexin.

Discussion

The antimicrobial susceptibility pattern of all the MRSA isolates in this experiment was determined by the Kirby Bauer disc diffusion method and has been shown in Table 5. It has found that all the isolates were resistant to penicillin. Approximately 68.8(%) isolates have found to be resistant to

Ciprofloxacin and 37.5% ampicillin, which is a cause for concern. About 63(%) isolates have found to be resistant to cotrimoxazole 68.8%, and the resistance to Gentamicin and cloxacillin ranged from 37-5% (Table 5). *Staphylococcus aureus* has long recognized as a significant human pathogen responsible for a wide range of infections, ranging from mild skin infections to wound infections and bacteremia. Although the introduction of antibiotics over the last 50 years has reduced the mortality rate from these infections, the bacteria have developed resistance mechanisms to all the available antimicrobial agents.

Table 5 Antibiotics susceptibility pattern of *S. aureus*.

Antibiotics	MRSA(n=16)		
	Sensitive	Intermediate	Resistance
Ciprofloxacin	4(25%)	1(6%)	11(68.8%)
Ampicillin	8(50%)	2(13%)	6(37.5%)
Cotrimoxazole	5(31.3%)	0(0%)	11(68.8%)
Getamicin	10(62.5%)	0(0%)	6(37.5%)
Cloxacillin	4(25%)	1(6%)	11(68.8%)
Cephalexin	6(35.7%)	1(6%)	9(56.3%)
vancomycin	16(100)	0(0%)	0(0%)

S. aureus is one of the most successful and adaptable human pathogens due to its proficiency in acquiring antibiotic-resistant mechanisms and pathogenic determinants, leading to its emergence in both nosocomial and community settings [16]. Diseases caused by *S. aureus* are a health hazard to humans worldwide. Since the first recognition of methicillin-resistant *S. aureus* in 1961 [17], there has been an upsurge of infections caused by the *S. aureus* variants that resist not only methicillin but also other -lactams and vancomycin, which are therapeutic drugs of choice [18], leading to treatment failure and increased case fatality rate. The methicillin and vancomycin resistance of the *S. aureus* has encoded by *staphylococcal* cassette chromosome mec (SCCmec) and *vanA*, respectively. In this study (Figure 4), the sensitive% of MRSA were Ciprofloxacin (8%), Ampicillin (15%), Cotrimoxazole (9%) Gentamicin (19%), Cloxacillin 8% Cephalexin (11%) and Vancomycin (30%). This study was carried out at Everest hospital to determine the prevalence of MRSA in clinically suspected samples. A total of 526 different clinical samples from admitted and Outpatients submitted to the microbiological laboratory were analyzed using standard microbiological procedures.

The emergence and spread of strains of *S. aureus* that are resistant to some first-line antibiotics are of public health importance. *Staphylococcus aureus* is innocuous in most environments but with remarkable adaptability and versatility, which has equipped it as a commensal and pathogenic organism. It is one of the most infectious agents with high prevalence in various community- and healthcare-associated infections. In this present study, the prevalence and

antibiogram of *S. aureus* isolates bacteriologically recovered. The isolates of *S. aureus* from clinical samples were categorized as MRSA by testing their susceptibility towards with Cefoxitin. The incidence of MRSA infection in this study was 26.6% based on significant bacterial growth. Higher growth rates were reported in various studies; 43.6% [12], 44.9% [13], 69.1% [14], 21.1% [15], 19% [16], 25.5% [9,17] in Nepal, 37% in India [18], 30.9% in Korea [19] and 51.0% in Pakistan [20]. However similar rates have been reported from other studies; 26.1% [21], 45.9% [22]. The difference in the rate of MRSA in different studies explained by differences in methodology used, the environment, indiscriminate use of antibiotics, difference in inclusion criteria of the samples, and the standard of personal hygiene and education.

The higher occurrence of *S. aureus* isolates in females reported in this study may be accounted for by the fact that the anatomical orientation of females exposes them to natural contamination by the bacterium since this pathogen is endogenously colonizing the vagina vault of healthy women [23]. However, the proximity of the urethral orifice to the rectum (which is in direct contact with perineal microbes) makes females more liable to urinary tract infection (UTI) due to *S. aureus*. The improper cleaning of the perineum, the use of napkins, and sanitary towel during the menstrual period could also be other predisposing factors that make females more prone to infection with the organism [23]. In males, the sterility of the proximal two-thirds of the urethra, it is a longer length, and the bactericidal effect of prostatic secretion constitutes an excellent immunological defense against bacterial infection. The resistance and susceptibility profile of the *S. aureus* isolates recovered in this study conforms to previous reports that *S. aureus* is notoriously resistant to some commonly used antimicrobial agents, including ampicillin and chloramphenicol which may clinically use for the treatment of infections caused by this organism [24].

Conclusion

The results of this study have shown that the *S. aureus* isolates recovered from apparently healthy pupils are resistant to some commonly used antibiotics. This study highlights the need for continuous surveillance of the occurrence and antibiogram of *Staphylococcus aureus* with the view to containing any disease outbreak due to them. The isolates were found predominantly in Pus and wounds followed by Sputum and Body fluids, and the incidence rate of Methicillin resistance *Staphylococcus aureus* (MRSA) is 26.6%. Antimicrobial susceptibility pattern shows that Vancomycin and Ampicillin were the most effective drugs against MRSA, and the isolates exhibited high and similar resistant (68.8%) to Ciprofloxacin, Cloxacillin, and Cotrimoxazole. The findings of the study conclude that MRSA is rising in number are seen more in inpatients. Vancomycin can use as a drug of choice for MRSA. The activity of all available drugs must be tested against the isolate to establish which ones could use to treat these infections, to control the dissemination of such multidrug-resistant bacteria. Treatment of multidrug-resistant MRSA is problematic because the choice of antibiotics in such cases in

limited; therefore, the MRSA can be controlled by regular monitoring and checking the irrational use of antibiotics.

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Author Contribution Statement

Shakti Regmi: Performed the experiments; Analyzed and interpreted the data; wrote the paper.

Jyoti Amatya: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools, or data; Wrote the paper.

Shyam Narayan Labh: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools, or data; Wrote the paper, processing, and corresponding for publication.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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