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## Prevalence of methicillin resistant *Staphylococcus aureus* in a tertiary hospital of Nepal

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### Abstract

**Introduction:** Methicillin resistant *Staphylococcus aureus* (MRSA) is one of the leading causes of healthcare associated infection. It causes different types of difficult-to-treat infections as these pathogens are resistant to  $\beta$ -lactam antibiotics. The changing epidemiology, ever-increasing prevalence and changing trends of susceptible antibiotics is a global concern. The aim of this study was to evaluate the prevalence of MRSA isolated from various samples.

**Method:** It was a hospital based descriptive cross-sectional study conducted at Patan Hospital, Patan Academy of Health Sciences. All the records of the patient whose culture and sensitivity report yielded *Staphylococcus aureus* from 1st January 2021 to 31st December of 2023 was extracted from the hospital electronic database and analyzed. Ethical approval was taken from the Institutional Review Committee.

**Result:** Out of 1259 *Staphylococcus aureus* isolates, 570(45.14%) were MRSA out of which, 380(66.67%) were identified from samples collected through outpatient department visits. MRSA had higher sensitivity to vancomycin (100%), linezolid (97.57%), doxycycline (93.17%) and chloramphenicol (84.67%). The sensitivity was concerning to clindamycin (39.57%), trimethoprim-sulfamethoxazole (36.23%), azithromycin (32.73%) and erythromycin (27.87%) and very alarming to gentamicin (19.00%), ofloxacin (5.57%) and ciprofloxacin (4.40%).

**Conclusion:** The prevalence of MRSA infection is alarming in the patients visiting our outpatient departments and has shown no signs of improvement over the past three years. Gradual decline to once sensitive drugs is now being observed. To address this issue, continuous surveillance, good Infection control practices and judicious antibiotic use are needed not only in hospitals but also in community settings.

**Keywords:** Antimicrobial Resistance; Healthcare Associated Infection; Methicillin; Multidrug; *Staphylococcus*



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## Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) are genetic sub-variants of the *Staphylococcus aureus* (*S. aureus*) containing *mecA* gene encoding penicillin-binding protein 2a (PBP 2a) which has low affinity for binding  $\beta$ -lactam antibiotics and hence confers the methicillin resistance.<sup>1</sup> Microbiologically, they are defined as having oxacillin minimum inhibitory concentration of greater than or equal to 4  $\mu\text{g}/\text{ml}$ .<sup>2-3</sup> MRSA is frequently resistant to most of the commonly used antimicrobial agents. It is one of the leading pathogens that causes of hospital-acquired infections and is commonly associated with significant morbidity, mortality, length of stay, and cost burden.<sup>2</sup>

Prevalence of MRSA has always been in increasing trend globally.<sup>4-5</sup> Initially, it was mostly restricted to hospital settings but MRSA has now also spread to the community.<sup>6-11</sup> In USA, MRSA rates increased from 2.1% in 1975 to 35% in 1991 and reached as high as 60% in certain U.S. centers.<sup>12-13</sup> Additionally, a report from Shanghai showed rates exceeding 70%, while in European centers, the rates ranged from 2% to 54.4%.<sup>14</sup> In Nepal, MRSA prevalence showed variation, ranging from 14.64% to 81.64%.<sup>15-16</sup> In 2019, MRSA caused more than 100,000 deaths.<sup>17-18</sup>

The changing epidemiology and high prevalence of MRSA is a serious concern and needs to be monitored continuously for possible deviation from usual antibiotic sensitivity patterns and warrant review of therapeutic approach. In this study, we evaluated the changes in prevalence and antibiogram of MRSA isolated from various samples at Patan Hospital, Patan Academy of Health Sciences from 1 January 2021 to 31 December 2023.

## Method

The study was conducted in the Microbiology unit of pathology and lab medicine department and record section of Patan Hospital, Patan Academy of Health Sciences, Lalitpur, Nepal. The unit processes approximately 45,000 culture and sensitivity tests every year on different clinical samples. It was a hospital based descriptive cross-sectional study where all the records of the patient whose culture and sensitivity report yielded *Staphylococcus aureus* from 1 January 2021 to 31 December 2023 was analyzed. The hospital microbiology laboratory identified *S. aureus* using conventional methods like inoculation of samples on 5% sheep blood agar, MacConkey agar and performing Gram's stain, catalase and coagulase tests on suspected *S. aureus* colony. Antimicrobial susceptibility testing (AST) was performed using

the modified Kirby Bauer disk diffusion method as recommended in the Clinical and Laboratory Standards Institute (CLSI; Wayne, PA, USA) guidelines and for vancomycin broth dilution for determination of minimum inhibitory concentration was used.<sup>19</sup> MRSA was detected using Cefoxitin disc (30  $\mu\text{g}$ ); a surrogate marker for oxacillin as detection of methicillin resistance using routine susceptibility test methods using oxacillin disc is known to be problematic.<sup>1</sup> WHO AWaRe (Access, Watch and Reserve group of antibiotics) classification for antibiotic stewardship was also followed when antibiotics were used for sensitivity testing and reporting.<sup>20</sup>

All demographic profiles and laboratory culture results of the patients were acquired from the electronic database of the hospital information system. Subsequently, the data underwent conversion from OpenDocument Spreadsheet (ODS) to Microsoft Excel format and a thorough data cleaning process. Various clinical samples were categorized into streamlined sample groups such as blood, respiratory (for example, sputum, bronchoalveolar lavage, tracheal aspirate, throat swab were all categorized into one respiratory sample) and urogenital, among others. Instances of multiple entries for the same patient within a 30-day period, each with a different laboratory number but featuring the same isolated organism, were meticulously addressed by eliminating duplicate entries within the EXCEL spreadsheet. The dataset encompassed antibiotic resistance patterns presented in tabulated form, alongside demographic data and unique inpatient hospital numbers.

Data were exported and analyzed using the statistical software Stata v15.1 (StataCorp, College Station, Texas, USA). Data are summarized using frequencies and percentages.

The study obtained ethical approval from the Institutional Review Committee of Patan Academy of Health Sciences, Lalitpur, Nepal (Ref: drs2403011842). As this study involved analyzing retrospective data from routine records stored in the hospital database system, the need for informed consent was waived; data confidentiality was maintained throughout the study.

## Result

The microbiology unit of Patan Hospital, Patan Academy Health Sciences received and processed 134116 different biological samples between the period of 1<sup>st</sup> January 2021 and 31<sup>st</sup> December of 2023 (34,169 in 2021; 48,958 in 2022 and 50,992 in 2023). Out of these, *S. aureus* was isolated from

1259 samples (356 in 2021, 490 in 2022 and 413 in 2023). For identification of methicillin resistance, all of those isolates were tested for resistance to oxacillin using surrogate marker (cefoxitin disc of 30 µg). The prevalence of MRSA (defined as resistance to oxacillin) among those with *S. aureus* infection was found to be 570(45.14%) of 1259 *Staphylococcus aureus* isolates during the three year period of data review. In 2021, the proportion was 157(44.10%) out of 356; 228(46.53%) out of 490 in 2022 and 185(44.79%) out of 413 in 2023), Table 1.

In those with *S. aureus* infection, the prevalence of MRSA was similar in males and females. In 2021, MRSA was isolated from 78(49.70%) males; 103(45.17%) males in 2022 and 81(43.78%) males in 2023. In 2021, MRSA was isolated from 79(50.30%) females; 125(54.82%) in 2022 and 104(56.21%) in 2023. Of the 570 MRSA isolates during the three-year period, 380(66.67%) were isolated from samples collected through out-patient department while 190(33.33%) were originated from the inpatient department. Out of 570 MRSA isolates, 431(75.61%) were pus samples followed by 86(15.09%) wound swabs, Table 2.

**Table 1. *Staphylococcus aureus* isolated from different biological specimen submitted to Microbiology unit of department of pathology and lab medicine at Patan Hospital (N=1259)**

Year	<i>Staphylococcus aureus</i> isolates (N)	MSSA* N(%)	MRSA N(%)
2021	356	199(55.89%)	157(44.10%)
2022	490	262(53.46%)	228(46.53%)
2023	413	228(55.20%)	185(44.79%)
Total	1259	686(54.85%)	570(45.14%)

\*MSSA: Methicillin Sensitive *Staphylococcus aureus*

**Table 2. Demographic profile of patients with MRSA isolated from different biological specimen submitted to Microbiology unit of department of pathology and lab medicine at Patan hospital (N=570)**

Demographics	2021, N(%) (N=157)	2022, N(%) (N=228)	2023, N(%) (N=185)
<b>Origin</b>			
IPD	30(19.10%)	102(44.70%)	58(31.40%)
OPD	127(80.90%)	126(55.30%)	127(68.60%)
<b>Sex</b>			
Female	79(50.30%)	125(54.82%)	104(56.21%)
Male	78(49.70%)	103(45.17%)	81(43.78%)
<b>Age Group, years</b>			
<1	7(4.45%)	7(6.79%)	19(10.27%)
1-5	14(8.91%)	20(19.41%)	21(11.35%)
6-18	18(11.46%)	15(14.56%)	24(12.97%)
19-35	67(42.67%)	28(27.18%)	60(32.43%)
36-50	20(12.73%)	18(17.47%)	26(14.05%)
51-65	19(12.10%)	8(7.76%)	20(10.81%)
>65	12(7.64%)	7(6.79%)	15(8.10%)
<b>Specimen</b>			
Pus	121(77.07%)	181(79.39%)	129(69.73%)
Wound swab	22(14.01%)	30(13.16%)	34(18.38%)
Blood	8(5.10%)	6(2.63%)	3(1.62%)
Respiratory sample	2(1.27%)	2(0.87%)	10(5.41%)
Body fluid	3(1.91%)	1(0.44%)	4(2.16%)
Uro-genital sample	1(0.64%)	6(2.63%)	1(0.54%)
Tissue	-	1(0.44%)	4(2.16%)
CSF	-	1(0.44%)	-
Urine	-	-	-
Catheter tip	-	-	-

**Table 3. Results of antibiotic susceptibility testing expressed in % sensitivity of MRSA isolated from biological samples of patients submitted to the microbiology laboratory at Patan Hospital (N=570)**

Antibiotics	AWaRe classification of antibiotics	Sensitivity (%)			Overall sensitivity
		2021	2022	2023	
Vancomycin	Watch	100	100	100	100.00
Linezolid	Reserve	98.10	97.80	96.80	97.57
Doxycycline	Access	92.80	94.30	92.40	93.17
Chloramphenicol	Access	75.20	86.40	92.40	84.67
Clindamycin	Access	47.80	32.50	38.40	39.57
Trimethoprim-sulfamethoxazole	Access	36.30	51.30	21.10	36.23
Azithromycin	Watch	36.60	28.10	33.50	32.73
Erythromycin	Watch	29.30	24.60	29.70	27.87
Gentamicin	Access	10.20	14.90	31.90	19.00
Ofloxacin	Watch	3.80	4.80	8.10	5.57
Ciprofloxacin	Watch	3.80	3.50	5.90	4.40

As per AWaRe classification of antibiotics by WHO<sup>20</sup>, all the *S. aureus* isolates were tested for trimethoprim-sulfamethoxazole, clindamycin, doxycycline, gentamicin and chloramphenicol belonging to the "Access group"; azithromycin, ciprofloxacin, ofloxacin, erythromycin and vancomycin belonging to the "Watch group" and linezolid belonging to the "Reserve group" of antibiotics.

For the antibiotics tested against MRSA, higher sensitivity was seen to Vancomycin (100%), linezolid (97.57%), doxycycline (93.17%) and chloramphenicol (84.67%). The sensitivity was concerning to clindamycin (39.57%), trimethoprim-sulfamethoxazole (36.23%), and azithromycin (32.73%) and erythromycin (27.87%) and very alarming to gentamicin (19%), ofloxacin (5.57%) and ciprofloxacin (4.40%), Table 3.

## Discussion

This study is a part of vigilant and continuous antimicrobial susceptibility surveillance keeping track of the changing antimicrobial resistance (AMR) profile on various organisms of interest in our hospital. MRSA, being one of the leading causes of pyogenic infection in hospital settings and now increasingly in community settings with no evidence related to exposure to healthcare, is one such organism of interest. The present article gives a review of the MRSA infections presented in Patan hospital and antimicrobial susceptibility from 2021 to 2023.

The prevalence of MRSA among those with *S. aureus* infections accounted at 45.14% (44.5% in 2021; 46.5% in 2022; 44.8% 2023), which is similar to the prevalence of Nepal 41.7%.<sup>15</sup> When comparing these findings to previous studies from Nepal, our result aligns closely to the findings of Mishra SK, et

al, Ansari S, et al, and Raut S et al, which reported MRSA prevalence rates of 42.4%, 43.1%, and 43.6% respectively.<sup>21-23</sup> However, these rates are lower than the 57% reported by Pradhan et al. from the same study setting as this study.<sup>16</sup> This decline in prevalence in this study could be due to robust infection control practices and antibiotic stewardships implemented in the hospital as a part of continuous improvement projects. However, the study also found that 66.67% of the biological samples with MRSA isolation originated from out-patient department services. This increasing level of MRSA isolation from samples originating from outpatient clinics suggests an increasing level of community acquired MRSA infection. These difficult to treat infections are of serious public health concerns and probably are attributed to non-judicious antibiotic use at community level in the country, poor infection control practices reaching the community level and poor microbiological reporting services where cefoxitin discs are not used to determine oxacillin resistance.

The infection did not vary among different genders but significant cases were seen in the age group of 19 to 35 years. This finding was similar to the previous study done by Pradhan et al. in the same setting.<sup>16</sup> It could be because this age group falls on the working group of the community in the country.

Although MRSA was isolated mainly from pus and wound swab accounting for more than 90% of the sample that yielded the isolation, our study did find isolation from other samples also. Hence a robust microbiological laboratory services is warranted in the country as the isolation of MRSA could be missed if standard techniques are not used.

Our study found that among the “access” group of antibiotics doxycycline (93.17%) and chloramphenicol (84.67%) were most sensitive. Doxycycline showed a stable trend and remained sensitive above 90% throughout 2021-2023 but it was noted that chloramphenicol showed a steady rise in sensitivity. It could be due to hesitancy to use chloramphenicol in general clinical practice and hence the resistance mechanism towards it is not expressed in *Staphylococcus* spp. However, cotrimoxazole which was sensitive in 71% of MRSA isolates in 2018-2020 study by Pradhan et al. is now showing sensitivity only at 36.23%.<sup>16</sup> This is a very serious situation as a cheap and easily accessible drug from access group with good coverage is now showing decrease in sensitivity. This could be hypothesized by gene mutation or development of any other resistance mechanism in MRSA isolates to cotrimoxazole and opens a new door for further research to find if any mutation has really occurred. Our study could not find any vancomycin resistant or intermediate isolates in the clinical isolates. Our study did find resistance to linezolid, a reserve group of antibiotics and more concerning was the fact that the sensitivity is gradually decreasing from 98.5% in 2021 to 96.8% in 2023.

This study was conducted on data produced using all the quality control rules for performance of antimicrobial sensitivity testing. All the *S. aureus* isolated were tested for oxacillin resistance using cefoxitin disc as surrogate marker. However, the gold standard for identifying MRSA is to detect the *mecA* gene, or its product, PBP2a, by latex agglutination. Most of the microbiology laboratories do not perform these molecular tests as these tests are relatively expensive and do not fall in the scope of routine microbiological testing. Detecting the *mecA* gene in future *S. aureus* isolates can be a new endeavor and might give a better insight on the prevalence at molecular level.<sup>24</sup> All the drug bug combination was used as per CLSI guidelines and inducible clindamycin resistance was also tested for all *S. aureus* isolate. A high volume of samples was analyzed during the study process with an isolation rate of more than 60% in pyogenic infection. With high isolation rate and good quality data, this study reflects clearly on the present prevalence rate and antibiotic sensitivity of MRSA. However, the reason for high isolation of MRSA from OPD source is limited and is an opportunity to look into. The prescribing clinicians should be encouraged to send more biological samples for microbiological studies as the increasing yield of isolates may put a clearer picture on the actual prevalence and antibiotic susceptibility. Additionally, increased surveillance through comprehensive microbiological studies enables healthcare facilities to implement targeted interventions and antimicrobial stewardship programs

aimed at mitigating the spread of antimicrobial resistance. Thus, a strong advocacy for the submission of biological samples for microbiological analysis is the key in combating the growing threat of antimicrobial resistance and ensuring effective patient care.

## Conclusion

Approximately 45% of all *S. aureus* strains isolated from biological samples at Patan Hospital exhibited resistance to oxacillin. Although consistent over the past three years, there is a notable decline in the prevalence in our hospital. The susceptibility of linezolid has exhibited a gradual decline over the years while cotrimoxazole has shown a marked decline in susceptibility. For the treatment of MRSA infections, Vancomycin and linezolid is to be considered. Additionally, Doxycycline and chloramphenicol can be an alternative approach of treatment. These drugs can be considered as initial treatment options for MRSA infections, with adjustments made based on available culture and sensitivity testing results.

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## Conflict of interest

None

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## Author's contribution

Concept, design, planning: all authors PR, SM, PP, SA; Literature review: PR, SM, PP, SA; Data collection/analysis: PR, PP; Draft manuscript: PR, SM, SA; Revision of draft: SM, PP, SA; Final manuscript: PR, SM, PP, SA; Accountability of the work: PR, SM, PP, SA.

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