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Research Article

A STUDY OF METICILLIN RESISTANT PATTERN ON CLINICAL ISOLATES OF *Staphylococcus aureus* IN TERTIARY CARE HOSPITALS OF POKHARA

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ABSTRACT

Meticillin-resistant *Staphylococcus aureus* (MRSA) has become one of the well-known etiologic agents for a wide variety of infections in both hospital and community settings. It is also a growing threat to the immunocompromised as well as to the general public. A total of 98 *S. aureus* isolates from 450 different human clinical specimens comprising pus, nasal swab, blood, urine and sputum were obtained at two tertiary care hospitals of Pokhara; Manipal Teaching Hospital (MTH) and Western Regional Hospital (WRH). Those isolates were then screened for meticillin resistance by the Kirby Bauer disc diffusion technique following aseptic procedures in Microbiology laboratory, WRH. Antibiotic susceptibility pattern of Meticillin sensitive *S. aureus* (MSSA) and Meticillin resistant *S. aureus* (MRSA) were studied by using antibiotic discs like cefoxitin (30mcg), oxacillin (1mcg), vancomycin (30mcg) and gentamicin (10mcg). 72.4% of the isolates were found to be MRSA while 27.6% were MSSA. Among them, very high resistance levels (87.8%) and (74.5%) were detected against oxacillin and cefoxitin while gentamicin and vancomycin recorded the least resistance levels i.e (25.5%) and (5.1%) respectively. High percentage of meticillin resistant isolates and occurence of vancomycin resistance among them which may refer to irrational use of antimicrobial agent, thus, necessitate implementation of good strategies for control of infection and use of antibiotics. Outcome of this study emphasizes the need for constant monitoring on the prevalence of MRSA and to help clinicians/doctors in the effective management and treatment of infections caused by *S.aureus.*

KEYWORDS: MRSA, Antimicrobial Susceptibility, Meticillin, Cefoxitin, Oxacillin, Vancomycin

INTRODUCTION

Staphylococcus aureus is a gram positive, coccid bacteria is a serious current health care concern [1]. This organism has maintained its role as one of the commonest human pathogens in both community-acquired and hospital-acquired infections. It is uniquely resistant to adverse conditions such as low water activity, high salt content, osmotic and pH stress and relatively high heat resistant. This may be the reasons, it has remained a major pathogen, colonizing and infecting humans to produce different disease.

Although this bacterium was discovered in 1880s, its effective treatment started in 1940s; penicillin was the only drug used for treatment of the infections caused by this bacterium. However of effective treatment by penicillin, in late 1940s and throughout the 1950s, *S.aureus* developed resistance mechanism to penicillin. Introduction of Meticillin was done in 1961 to treat

these resistant strains infections and within a year or later, clinicians had encountered meticillin-resistant *S. aureus,* which became a big threat. Today, there are strains of MRSA that simultaneously resist a list of different antibiotics, including vancomycin which is often considered our last line of antibacterial defense.

Staphylococci has developed several mechanisms of meticillin resistance, including inactivation by the beta lactamase enzymes, penicillin binding proteins (PPB) with reduced penicillin binding capacity, and acquisition of the mec-A gene which encodes new penicillin binding proteins PBP-2a with low affinity for beta lactams. The later mechanism accounts for the majority of resistance to meticillin and other beta lactams.[2] MRSA strain has become one of the leading etiologic agents of a wide variety of infections throughout the world. Health care-associated MRSA and community associated MRSA are growing together as a threats to the immunocompromised, as well as to the general public health [1]. Accurate diagnosis of meticillin-resistance in *S. aureus* is one of the major importances to ensure effective treatment for the affected patient and to prevent further transmission. After the first case of MRSA arose back in UK (1961), there has been an increasing rate of infections caused by MRSA worldwide resulting in increased mortality and morbidity rate. Considering the increasing statistics of infections caused by MRSA, there is the requirement of reliable, accurate and rapid testing for detection of MRSA is essential for both antibiotic therapy and infection control measures. A specific detection of MRSA is crucial not only for early prevention of disease spread, but also for the effective treatment of these infections. Clinician's require rapid and accurate identification of MRSA to select appropriate antibiotic treatment and to avoid the transmission of these strains. However, due to limitation of optimal phenotypic method for detecting meticillin resistance in *S.aureus*, and genotypic tests involving mec A gene detection by PCR, which are considered to be the reference, are not practical and applicable for routine use in clinical laboratories.[3] This remains a need for a reliable testing procedure for MRSA detection that can be performed easily in routine situations.[4]

This study was performed for the constant monitoring on the prevalence of MRSA and its microbial susceptibility pattern. Outcome of this study would help clinicians/doctors in the effective management and treatment of health care associated infections caused by *S.aureus.*

OBJECTIVES

General objective

i. To know about the resistance pattern of MRSA in tertiary hospitals of Pokhara.

Specific objectives

i. To compare the resistance pattern of MRSA in two tertiary hospitals.

ii. To find out the resistance of MRSA among different age groups of people.

iii. To find out the resistance pattern of MRSA among sex groups.

iv. To identify vancomycin resistant strains of *S.aureus.*

v. To find out the distribution of MRSA isolates in different samples.

METHODS AND METHODOLOGY

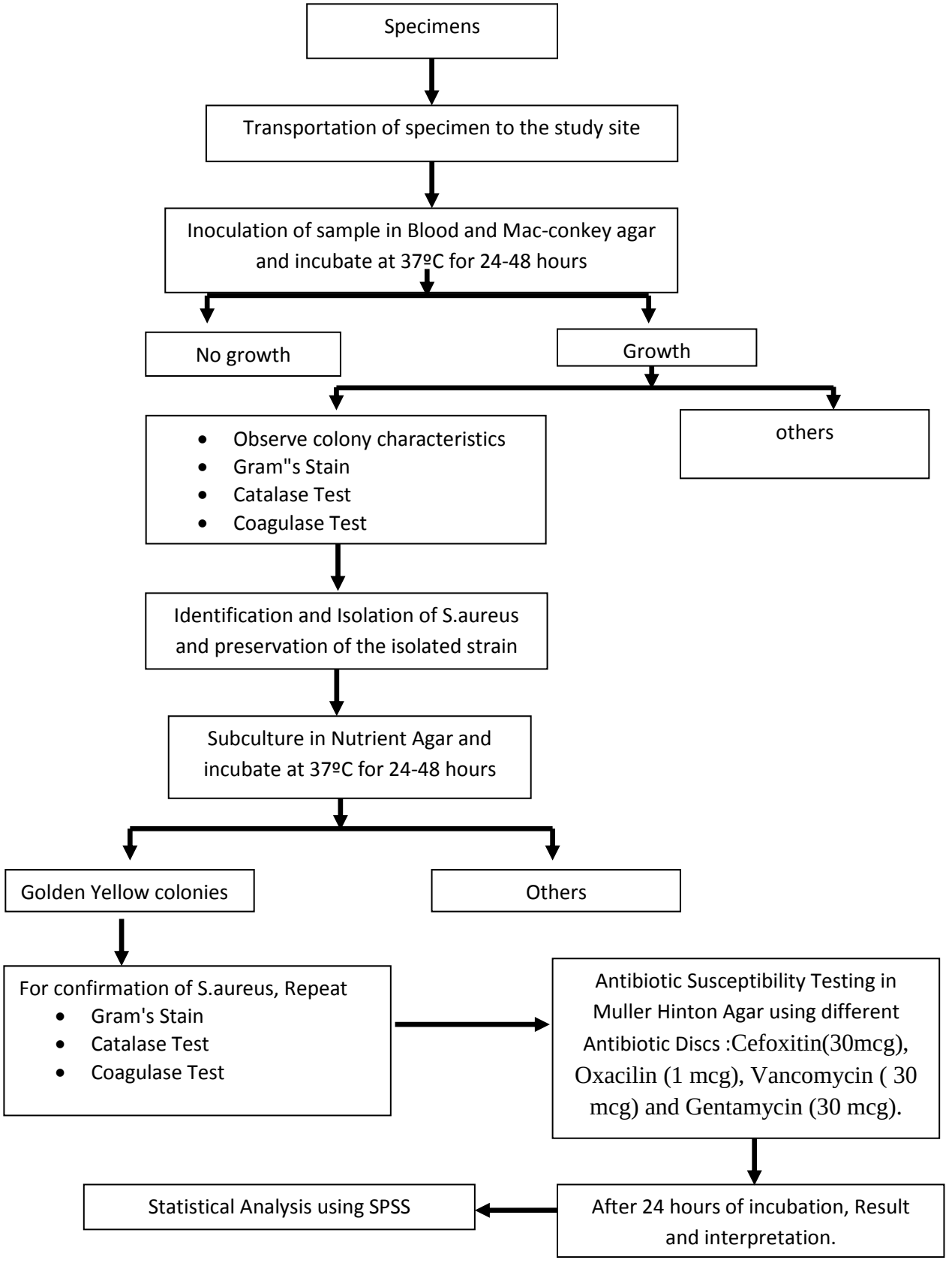
A hospital based cross-sectional study was carried out at two major tertiary care hospitals of Kaski Western Regional Hospital and Manipal Teaching Hospital, from 18th January to 8th March, 2016. Different body fluids sample was collected and was processed in Microbiology department of Pokhara University. The permission was taken from the School of Health Sciences, Pokhara University. The Consent for sample collection was taken from the Head of department of Microbiology at Western Regional Hospital and Manipal Teaching Hospital. The consent was also taken from each patient included in the study regarding the details of the research work and privacy of their identity for the further dissemination of results.

Only the isolates of *S.aureus* from all body fluids and of all age groups and gender were included. Duplicate isolates from the same patient were excluded in the study.

Clinical samples such as pus, wound swabs, urine, blood, sputum and nasal swabs were collected from patient visiting WRH and MTH. Samples were collected asceptically using standard procedure in a sterile container. Each sample was labelled with unique code number and various other information including age, sex, clinical history, location, etc were also recorded. Those samples were asceptically transported to Microbiology laboratory of WRH.

All samples were processed by inoculation in blood and Mac-conkey agar and S. aureus isolates were confirmed by gram staining, catalase test, coagulase test and followed by antibiotic sensitivity test using different antibiotics like Cefoxitin (30mcg), Oxacilin (1 mcg), Vancomycin (30 mcg) and Gentamicin (30 mcg) on Muller Hinton Agar and the results were noted.

RESEARCH DESIGN



RESULTS

A total of 450 samples including pus, blood, sputum, urine and nasal swab were collected using standard procedure from Western Regional Hospital (WRH) and Manipal Teaching Hospital (MTH) and transported to the study site in an ascetical conditions. Those samples were inoculated in Blood Agar (BA) and Macconkey Agar (MA) and on the basis of Gram's stain; Slide Coagulase Test, *S.aureus* isolates were identified, isolated and preserved in special media. Later the preserved isolates were sub-cultured on Nutrient Agar (NA).

Sample distribution from Inpatient and Outpatient

A total of 98 Staphylococcus were isolated from different samples of inpatient and outpatient of WRH and MTH. These included 37 (37.8%) inpatient and 61 (62.8%) outpatient.

Table 1: Sample distribution from Inpatient and Outpatient

Name of the Hospitals	Sex of the patients	Indoor and outdoor patients		Total
		Indoor patients	Outdoor patients	
Western Regional Hospital	Male	9	10	19
	Female	10	5	15
Manipal Teaching Hospital	Male	8	27	35
	Female	10	19	29
Total		37	61	98

Age-wise distribution of samples

Out of total 98 isolates of *S.aureus*, 14 (14.3%) were observed in infants, 12 (12.2%) isolates were observed in children, in age group 15-45, 41 (41.8%) isolates. Likewise, 31 (31.6%) *S.aureus* were isolate in age group ≥45..

Table 2: Age-wise distribution of sample

Age of the patients	Type of samples					Total
	Pus	Nasal Swab	Blood	Urine	Sputum	
≤1	3	0	11	0	0	14
1-15	4	4	2	2	0	12
15-45	14	21	1	4	1	41
≥45	7	15	1	3	5	31
Total	28	40	15	9	6	98

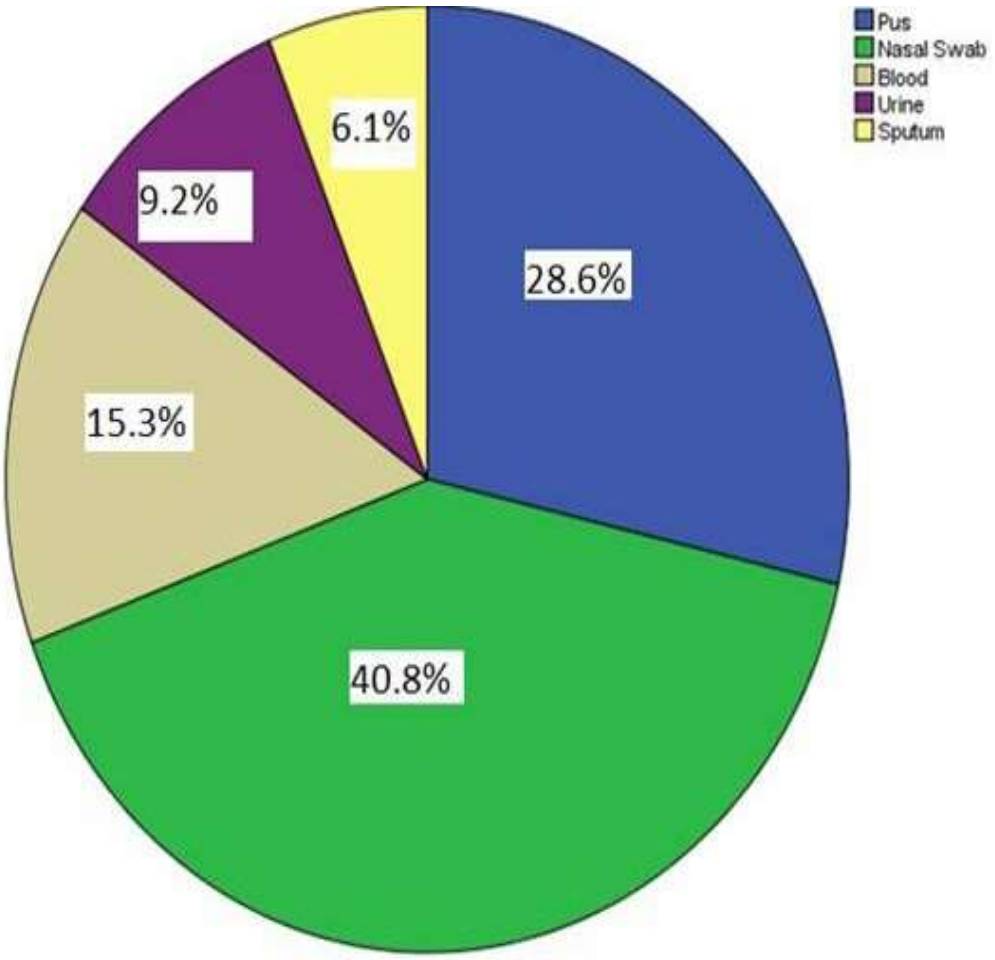
Distribution of *S.aureus* in Different Samples

Out of 98 *S.aureus* isolates, 71 were identified as MRSA by cefoxitin and or oxacilli. The high occurrence of MRSA was found in pus (20) and nasal swab (26).

Table 3: Distribution of S.aureus in Different Samples

Sample	MRSA			MSSA		
	Inpatient	Outpatient	Total	Inpatient	Outpatient	Total
Pus	9	11	20	5	3	8
Nasal swab	15	11	26	6	8	14
Blood	0	13	13	0	2	2
Urine	0	7	7	0	2	2
Sputum	1	4	5	1	0	1
Total	25	46	71	12	15	27

Figure 1: Figure showing frequency of S.aureus from different sample types



Age-wise Distribution of MRSA and MSSA

Out of 98 *S.aureus* isolates, 54 were from male and 44 were from female. The highest number (30) was isolated from the age group 15-45 years with second highest (22) from the age group more than 45 years.

Table 4: Age-wise Distribution of MRSA and MSSA

Age group	Male			Female		
	MRSA	MSSA	Total	MRSA	MSSA	Total
< 1 year	8	1	9	5	0	5
1-14 years	3	3	6	3	3	6
15-45 years	12	4	16	18	7	25
> 45 years	18	5	23	4	4	8
Total	41	13	54	30	14	44

Hospital-wise Distribution of MRSA and MSSA

Out of 98 *S.aureus* isolates, 37 were from Inpatient Door and 61 were from Outpatient Door. Out of 71 MRSA, the highest number (33) was isolated from Outpatient Door of Manipal Teaching Hospital.

Table 5: Hospital-wise Distribution of MRSA and MSSA

Name of the Hospital	Inpatient			Outpatient		
	MRSA	MSSA	Total	MRSA	MSSA	Total
Western Regional Hospital	13	6	19	13	2	15
Manipal Teaching Hospital	12	6	18	33	13	46
Total	25	12	37	46	15	61

Antibiotic sensitivity pattern for vancomycin

Out of 98 vancomycin (30mcg) discs used to test 98 isolates, only 5 (5.1%) MRSA isolates showed resistance and 93 (94.9%) isolates (66 MRSA and 27 MSSA) showed positive sensitivity to vancomycin.

Table 6: Antibiotic sensitivity pattern for vancomycin

MRSA positive/ negative	Antibiotic Sensitivity Testing for Vancomycin		Total
	Resistant	Sensitive	
MRSA	5	66	71
MSSA	0	27	27
Total	5	93	98

Antibiotic Susceptibility Pattern of *S.aureus* strains in different clinical samples

Among 98 cefoxitin(30mcg) discs used, 73 (74.5%) isolates showed resistance whereas, 25 (25.5%) showed sensitivity, similarly oxacillin (1mcg) discs used, 86(87.8%) isolates showed resistance whereas, 12 (12.2%) showed sensitivity. Among 98 vancomycin(30mcg) discs used,5 (5.1%) isolates showed resistance whereas, 93(94.9%) showed positive sensitivity. Lastly, Among 98 gentamicin (10mcg) discs used, 25 (25.5%) isolates showed resistance whereas, 73 (74.5%) showed sensitivity respectively.

Table 7: Antibiotic Susceptibility Pattern of S.aureus strains in different clinical samples

Sample Type	Cefoxitin		Oxacillin		Vancomycin		Gentamicin	
	R	S	R	S	R	S	R	S
Pus	20	8	24	4	2	26	8	20
Nasal swab	27	13	35	5	1	39	8	32
Blood	13	2	14	1	1	14	4	11
Urine	7	2	8	1	1	8	3	6
Sputum	6	0	5	1	0	6	2	4
Total	73	25	86	12	5	93	25	73
(%)	74.5	25.5	87.8	12.2	5.1	94.9	25.5	74.5

DISCUSSION

The prevalence rate of MRSA in the present study was found to be 72% which is in accordance with the study conducted in Chitwan, Nepal which has the prevalence of 68%.[5] Similar finding was seen in a study conducted in Western part of Nepal i.e. 69.1%.[6] While on contrary, the prevalence of MRSA among clinical specimens in Kathmandu Medical College ,Teaching Hospital was found to be 26.12% [7] which is less than the prevalence rate observed in the present report. Similarly, a study conducted in Manipal Teaching Hospital, Pokhara found the prevalence of MRSA to be 20%. [8]Likewise, the study of MRSA from nasal swab among school children conducted in Pokhara found MRSA prevalence of 56.1%.[9] The prevalence of MRSA acquired from our study is an indication of the increasing trend of MRSA in Nepal.

The prevalence of MRSA was higher in WRH (76%) than in MTH (70%) which is in agreement to the study conducted in two Nepalese Tertiary care hospitals showing that Kathmandu Based Hospital (52.3%) has higher prevalence of MRSA than Lalitpur Based Hospital (38%) [10] This may be due to the reason that the WRH is a big and busy hospital with a remarkable load of referral cases.

In the present study, the prevalence of MRSA was found higher in males (41%) than in females (30%) Similar findings were illustrated in a study conducted in Chitwan, Nepal where the prevalence of MRSA was 75% and 63.4% among males and females respectively.[11]

In the present study, the prevalence of MRSA is higher among the age group of 15-45 i.e. 30.6% which correlates with the study carried out in two tertiary care hospitals namely KBH and LBH which showed respective prevalence of 52% and 44% in similar age groups.[10]

In the present study, all the isolates of *S.aureus* were meticillin resistance in collected urine samples i.e. 9 MRSA out of 9 isolated *S.aureus*. Similarly, a total of 6 *S.aureus* were isolated from collected sputum sample, out of which 5 were identified as MRSA (5 MRSA out of 6 *S.aureus* isolates) which is higher than the previously reported studies in Tertiary-Care Hospital in Eastern Nepal (6 in 10 isolates)[12] and India (1 in 6 isolates).[13] This demonstrates an increasing trend from the reported previous studies indication that MRSA possesses a possible threat for UTIs and RTIs respectively in upcoming days.

In the present study, out of 71 MRSA 5 were identified as VRSA which is a prevalence rate of 7.04% which is contradictory to the studies in tertiary care of Hyderabad, India (2.46%). Similarly, studies carried out in tertiary care hospitals in eastern Nepal [12] Manipal

Teaching Hospital [8] and Kathmandu Medical College-Teaching Hospital [14] no VRSA was identified among 196, 20 and 111 isolated *S.aureus* from different specimens. This is a very important finding that alarms a need for an approach to find agents that can reduce or moderate resistance to an existing antibiotic.

CONCLUSION AND RECOMENDATION

Among 450 different clinical samples collected from two tertiary care hospitals (Western Regional Hospital and Manipal Teaching Hospital), 98 *Staphylococcus aureus* were isolated in which 71 were MRSA with prevalence rate of 11.83% of total cases. MRSA tend to emerge higher in age group 15-45 years (30.6%). The highest rate of prevalence of MRSA was found to be 26.5% in nasal swab. Our study also showed 5 Vancomycin Resistant *Staphylococcus aureus* (VRSA) and the emergence of VRSA after MRSA might be prevalent in other parts of the country as well, as antibiotic misuse is equally common.Hence, there should be an immediate response from the concerned authorities to check further emergence and spreading of these notorious MRSA as well as VRSA strains. A strict regulation on irrational antibiotic usages might be an appropriate and effective approach in this direction. Moreover, nationwide surveillance program should be carried out to map the meticillin and vancomycin susceptibility pattern in the country.

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