



TO STUDY THE PREVALENCE OF MRSA AND ITS ANTIMICROBIAL SUSCEPTIBILITY PATTERN AMONG STAPHYLOCOCCUS AUREUS ISOLATED FROM VARIOUS CLINICAL SAMPLES

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ABSTRACT

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a common nosocomial infection that causes severe morbidity and mortality. MRSA (methicillin-resistant *S. aureus*) is now endemic in India. In India, the prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) ranges between 30 to 70%, resulting in high mortality, increased economic burden, and treatment failure in tertiary care facilities. Rapid and consistent MRSA detection is crucial for infection control and preventing unnecessary antibiotic use.

Aim and Objective: This study aimed to find out the prevalence of MRSA and to assess antibiotic activity against methicillin-resistant *Staphylococcus aureus* in vitro.

Material and Methods: This was a Cross-sectional observational study carried out in the Department of Microbiology at Government Medical College, Kota, Rajasthan for a period of three years, starting from September 7, 2019, to September 6, 2022. A total of 384 *Staphylococcus aureus* isolated from different clinical samples were analyzed. MRSA isolates were screened and confirmed using standard methods recommended by the Clinical and Laboratory Standards Institute (CLSI). Methicillin resistance, in *Staphylococcus aureus* strains, was evaluated using cefoxitin. The Kirby-Bauer disc diffusion technique was used to assess the antibiotic susceptibility pattern of all MRSA strains. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method according to the CLSI guidelines 2019.

Results: In the present study a total of 384 *Staphylococcus aureus* isolates were obtained from various clinical samples. The prevalence of MRSA was observed to be 48.1%. Among 384 *S. aureus* isolates, 70 (18.23%) belonged to (21-30) age group followed by 67 (17.45%) and least was observed in the age group 15 (3.91%) from (0-10) age group. The ratio of males was more compared to the females 195 (50.7%), 189 (49%) respectively in case of *S. aureus* and for MRSA Male was observed to be 98 (52%), female 87 (47.2%) respectively. The maximum number of isolates were observed for pus followed by urine and least for pleural fluid. It was also noted that Vancomycin was found to be the most sensitive with the sensitivity of 97.3%, followed by Linezolid 96.2% and then Teicoplanin 89.73 % was found to be the most effective drug of choice.

Conclusion: The prevalence of MRSA varies according to geographical region, hospital type, examined population, and detection technique. Effective antimicrobial activity as well as cost effectiveness should be considered in drugs prescribed for MRSA infections. Given the clinical implications of MRSA infection and its rapid transmission potential, MRSA strains must be evaluated on a frequent basis.

Keywords: MRSA, Disc Diffusion, CLSI, Prevalence, AST

INTRODUCTION

The *Staphylococcus* genus includes pathogenic organisms, with *Staphylococcus aureus* being the most prevalent. It has outperformed most treatment medicines discovered against it in recent years [1]. In the early 1960s, beta-lactamase resistant semi-synthetic penicillin's gave temporary respite. However, MRSA emerged shortly after methicillin became accessible for clinical use [2].

Methicillin resistance was first detected in *S. aureus* in 1961, [3,4] shortly after the agent was introduced clinically and over the last four decades, there has been a global epidemic of methicillin-resistant *S. aureus* (MRSA). [5-7] MRSA is usually acquired during exposure to hospitals and other healthcare facilities and causes a variety of serious healthcare-associated infections. The problem is exacerbated by the propensity of the organism to cause cross-infection and its ability to colonize individuals for months or years. Considerable selection pressure for this organism is applied in the hospital setting due to the now intensive use of the many antibiotics, particularly cephalosporins, to which the organism is resistant.

Many of these MRSA isolates are becoming multidrug resistant and are susceptible only to glycopeptide antibiotics such as vancomycin. Low level resistance even to vancomycin is emerging at present [8]. A significant obstacle that must be overcome in clinical settings is the presence of vancomycin resistance in *Staphylococcus aureus* (VRSA). Vancomycin, which is a glycopeptide antibiotic, has been considered for a long time to be the last line of defense against severe infections that are caused by methicillin-resistant *Staphylococcus aureus* (MRSA). On the other hand, the effectiveness of this essential antibiotic is being undermined by the appearance and proliferation of VRSA strains. In most cases, VRSA strains develop resistance by acquired genetic pathways, which often include the acquisition of *vanA* or *vanB* genes. Because these genes generate altered cell wall precursors, the binding affinity of vancomycin to its target site is decreased. As a result, the antibiotic is unable to effectively suppress the development of bacteria. Furthermore, vancomycin-intermediate *Staphylococcus aureus* (VISA) strains have been discovered. These bacteria have a decreased sensitivity to vancomycin, but they do not fulfill the requirements for complete resistance [9,10].

Prolonged hospital stays, indiscriminate use of antibiotics, lack of awareness, and receipt of antibiotics before coming to the hospital are some of the possible predisposing factors of MRSA emergence. The knowledge of MRSA prevalence and the current antimicrobial profile is necessary in selection of appropriate empirical treatment of these infections [9]. Control of MRSA in hospitals is essential. This can be achieved by proper implementation of hospital infection control measures and regular surveillance activity. MRSA infection, stressing the need for precision and promptness in MRSA identification in the clinical setting for the timely management of infections brought on by this superbug [10-12].

Furthermore, the development of resistance mechanisms and the spread of resistant strains within healthcare settings and communities may be better understood by the ongoing monitoring of resistance trends over time. This gives useful information. Early diagnosis of developing resistance patterns enables prompt actions, such as revisions in antibiotic prescription practices, deployment of infection control measures, and focused antimicrobial stewardship activities aimed at conserving the efficacy of current medicines [13,14].

Given the significance of MRSA infections for public health, it is essential to track the present scenario of MRSA prevalence, as part of antimicrobial resistance (AMR) surveillance, across a

variety of health care settings. Therefore, the present study was undertaken to study the prevalence and its antibiogram profile of MRSA isolates from various clinical samples.

MATERIAL AND METHODS

This was a cross sectional study carried out among the patients (inpatients and outpatients) conducted in the Department of Microbiology.

Data Collection and Procedure

Place of Study:The study was conducted in the Department of Microbiology, Government Medical College, Kota, Rajasthan, India.

Duration of Study: Data collection took place over a period of three years, starting from September 7, 2019, to September 6, 2022, following approval from the Departmental Research Committee (DRC).

The Ethical Letter: The Ethical clearance was duly obtained from the Institutional Medical College of GMC, Kota.

Statistical Analysis

Statistical analysis may include calculations of prevalence rates, descriptive statistics of sample characteristics, and comparisons of antimicrobial susceptibility patterns among different clinical samples. Additionally, the correlation between demographic variables and antibiotic resistance profiles may be analyzed using appropriate statistical tests. The significance level for statistical tests may be set at $p < 0.05$.

A total of 384 non repeated pus, wound swab, blood, nasal swab, throat swab, urine etc clinical samples from different clinical samples were received from the patients for bacteriological culture were included in the study.

Isolation and Identification of *Staphylococcus aureus*

The samples were inoculated on blood agar and mannitol salt agar (HiMedia laboratories private limited, India) and incubated aerobically at 37°C for 48 hours. The strains of *Staphylococcus aureus* were identified on the basis of colony morphology, Gram's stain, and different biochemical tests [15].

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion technique using Mueller-Hinton agar (HiMedia laboratories private limited, India) following Clinical and Laboratory Standards Institute (CLSI) guidelines 2019 [15]. Identified morphologically and biochemically by standard laboratory procedures including tube coagulase test and DNase test using DNase agar (Oxoid Ltd, Basingstoke, Hampshire, England).

Table No. (A): Antibiotic discs used in this study

Sr. No.	Antibiotics	Conc. (µg)	Zone of inhibition (mm)		
			Resistant	Intermediate	Sensitive
1	Amikacin	30	≤14	15-16	≥17
2	Cefazolin	30	≤19	20-22	≥23
3	Cefepime	30	≤18	19-24	≥25
4	Cotrimoxazole	25	≤10	11-15	≥16
5	Cloxacillin	5	≤21	22-24	≥25
6	Clindamycin	10	≤14	15-20	≥21

7	Cefoperazone	75	≤16	17-21	≥22
8	Erythromycin	15	≤13	14-22	≥23
9	Levofloxacin	5	≤15	16-18	≥19
10	Tetracycline	30	≤14	15-18	≥19
11	Quinupristin-Dalfopristin	15	≤15	16-18	≥19
12	Teicoplanin	30	≤10	11-13	≥14
13	Linezolid	30	≤20	-	≥21
14	Vancomycin	30	≤14	15-16	≥17
15	Nitrofurantoin	300	≤14	15-16	≥17

Detection of Strains of MRSA by Cefoxitin Disc Diffusion Method

Susceptibility of *Staphylococcus aureus* isolates to cefoxitin (30µg) was determined by Kirby-Bauer disc diffusion method following CLSI guidelines [15]. The strains of *Staphylococcus aureus* which were found to be resistant to cefoxitin were screened as MRSA.

RESULTS

In the present study a total of 384 *Staphylococcus aureus* isolates were obtained from various clinical samples among the patients admitted.

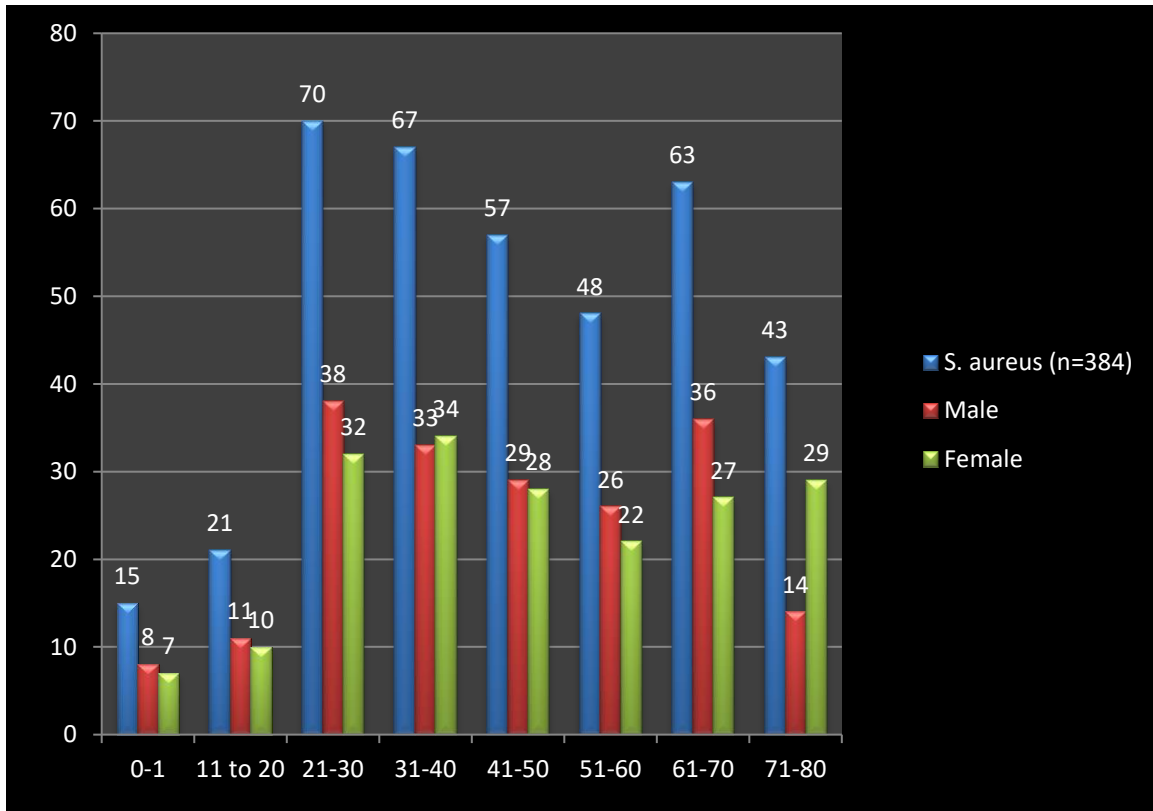
Table No. 1: Age and sex wise distribution of *Staphylococcus aureus* (n = 384).

Sr. No.	Age group	<i>S. aureus</i> No. (%)	Male No. (%)	Female No. (%)
1	0-10	15 (3.91)	8 (53.33)	7 (46.67)
2	11-20	21 (5.47)	11 (52.38)	10 (47.62)
3	21-30	70 (18.23)	38 (54.29)	32 (45.71)
4	31-40	67 (17.45)	33 (49.25)	34 (50.75)
5	41-50	57 (14.84)	29 (50.88)	28 (49.12)
6	51-60	48 (12.50)	26 (54.17)	22 (45.83)
7	61-70	63 (16.41)	36 (57.14)	27 (42.86)
8	71-80	43 (11.20)	14 (32.56)	29 (67.44)
	Total	384 (100)	195 (50.78)	189 (49.22)

Among 384 *S. aureus* isolates, 70 (18.23%) belonged to (21-30) age group followed by 67 (17.45%) from (31-40) age group, 63 (16.41%) from (61-70) age group, 57 (14.84%) from (41-50) age group, 48 (12.50%) from (51-60) age group, 43 (11.20%) from (71-80) age group, 21 (5.47%) from (11-20) age group, 15 (3.91%) from (0-10) age group.

Among 195 *S. aureus* isolated from male patients, 38 belonged to (21-30) age group followed by 36 from (61-70) age group, 33 from (31-40) age group, 29 from (41-50) age group, 26 from (51-60) age group, 14 from (71-80) age group, 11 from (11-20) age group, 8 from (0-10) age group.

Among 189 *S. aureus* isolated from female patients, 34 belonged to (31-40) age group followed by 32 from (21-30) age group, 29 from (71-80) age group, 28 from (41-50) age group, 27 from (61-70) age group, 22 from (51-60) age group, 10 from (11-20) age group, 7 from (0-10) age group.

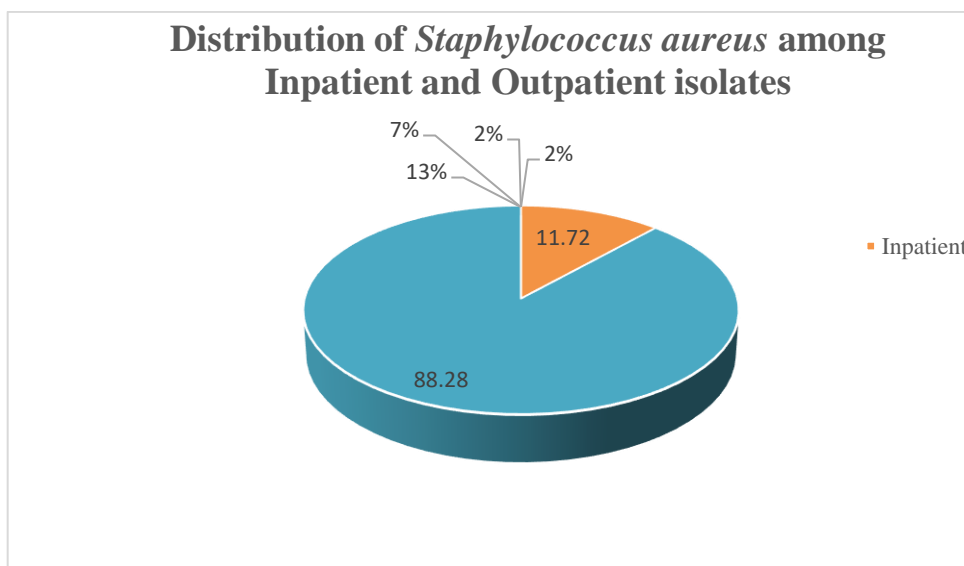


Graph No.1: Graphical Representation of Age and Sex Wise Distribution of *Staphylococcus aureus* (n = 384)

Table No. 2: Distribution of *S. aureus* isolates obtained from various clinical samples according to Inpatient and Outpatient isolates.

<i>S. aureus</i> No. (%)	Inpatient isolates No. (%)	Outpatient isolates No. (%)
384 (100)	45 (11.72)	339 (88.28)

Out of 384 (100%) *S. aureus* isolates, 339 (88.28%) were from outpatient isolates and 45 (11.72%) were from inpatient isolates.

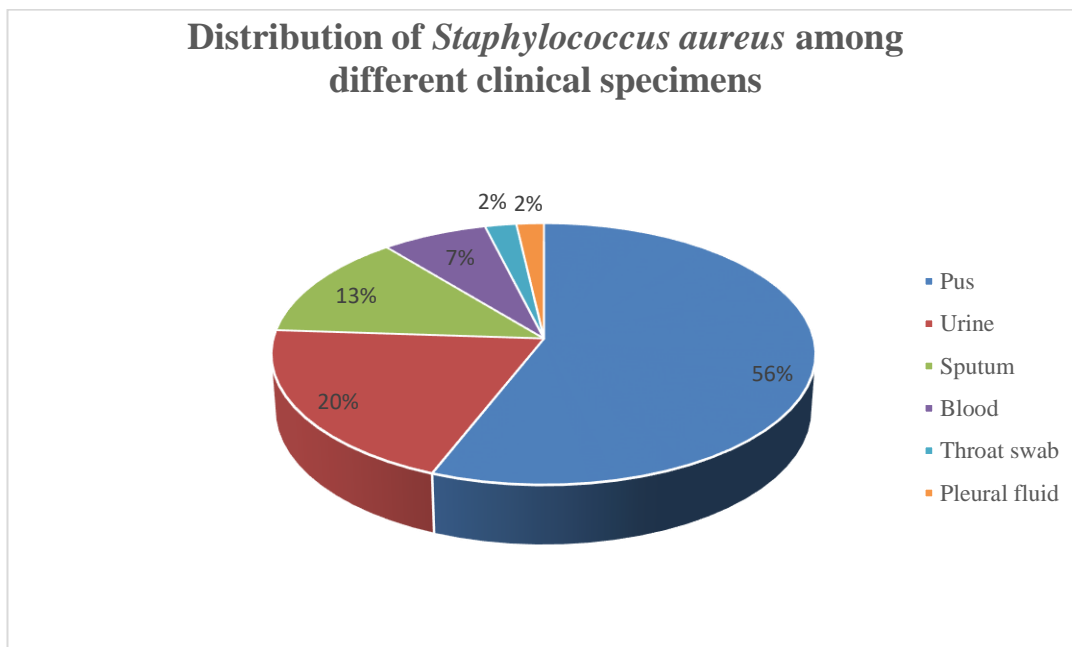


Graph No. 2: Graphical Representation of Distribution of *S. aureus* isolates among Inpatient and Outpatient isolates.

Table No. 3: Distribution of *S. aureus* isolates obtained from various clinical specimens.

Sr. No.	Clinical Specimens	Number of <i>Staphylococcus aureus</i>	Percentage (%)
1	Pus	215	56
2	Urine	77	20
3	Sputum	50	13
4	Blood	27	7
5	Throat swab	8	2
6	Pleural fluid	7	2
	Total	384	100

In the present study the highest percentage of *Staphylococcus aureus* isolates was obtained from pus samples (56%), followed by urine (20%), sputum (13%), blood (7%), throat swab (2%) and pleural fluid (2%).



Graph No.3: Distribution of *Staphylococcus aureus* among different clinical specimens.

Table No.4: Gender wise distribution of *S. aureus* isolates obtained from various clinical samples.

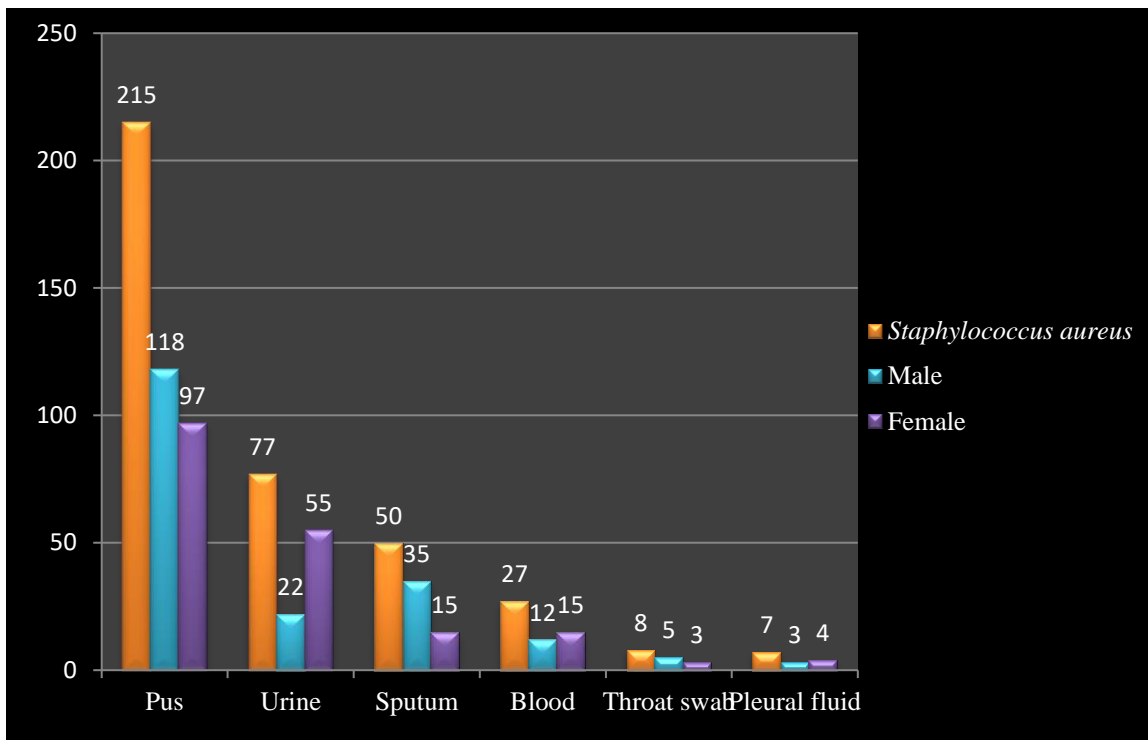
Sr. No.	Clinical Specimens	Male (%)	Female (%)
1	Pus	215 (55.99)	118 (54.88)
2	Urine	77 (20.05)	22 (28.57)
3	Sputum	50 (13.02)	35 (70)
4	Blood	27 (7.03)	12 (44.44)
5	Throat swab	08 (2.08)	05 (62.50)
6	Pleural Fluid	07 (1.82)	03 (42.86)
	Total	384 (100)	195 (50.78)

Male patients:

Out of 195 (50.78%) isolates from male patients, 118 were from pus followed by sputum 35, urine 22, blood 12, throat swab 5 and pleural fluid 3.

Female subjects:

Of the isolates 189 (49.22%) from the clinical samples of the female subjects, 97 were from pus followed by urine 55, sputum 15, blood 15, pleural fluid 4 and throat swab 3.



Graph No.4: Gender wise distribution of S. aureus isolates obtained from various clinical samples.

Table No. 5: Age group and sample wise distribution of S. aureus isolates.

Sr. No.	Age group	S.aureus N (%)	Pus N (%)	Urine N (%)	Sputum N (%)	Blood N (%)	Th.S. N (%)	P. Fluid N (%)
1	0-10	15 (3.91)	07 (46.67)	02 (13.33)	00 (0)	05 (33.33)	01 (6.67)	00 (0)
2	11-20	21 (5.47)	12 (57.14)	05 (23.81)	02 (9.52)	02 (9.52)	00 (0)	00 (0)
3	21-30	70 (18.23)	45 (64.29)	13 (18.57)	07 (10.00)	02 (2.86)	02 (2.86)	01 (1.43)
4	31-40	67 (17.45)	41 (61.20)	15 (22.39)	06 (8.96)	04 (5.97)	01 (1.49)	00 (0)
5	41-50	57(18.84)	32 (56.14)	11 (19.30)	07 (12.28)	05 (8.77)	01 (1.75)	01 (1.75)
6	51-60	48 (12.50)	27 (56.25)	09 (18.75)	08 (16.67)	03 (6.25)	00 (0)	01 (2.08)
7	61-70	63 (16.41)	30 (47.62)	12 (19.05)	13 (20.63)	04 (6.35)	02 (3.17)	02 (3.17)
8	71-80	43 (11.20)	21 (48.84)	10 (23.26)	07 (16.28)	02 (4.65)	01 (2.33)	02 (4.65)
	Total	384	215	77	50	27 (7.03)	08 (2.08)	07 (1.82)

		(100)	(55.99)	(20.05)	(13.02)		
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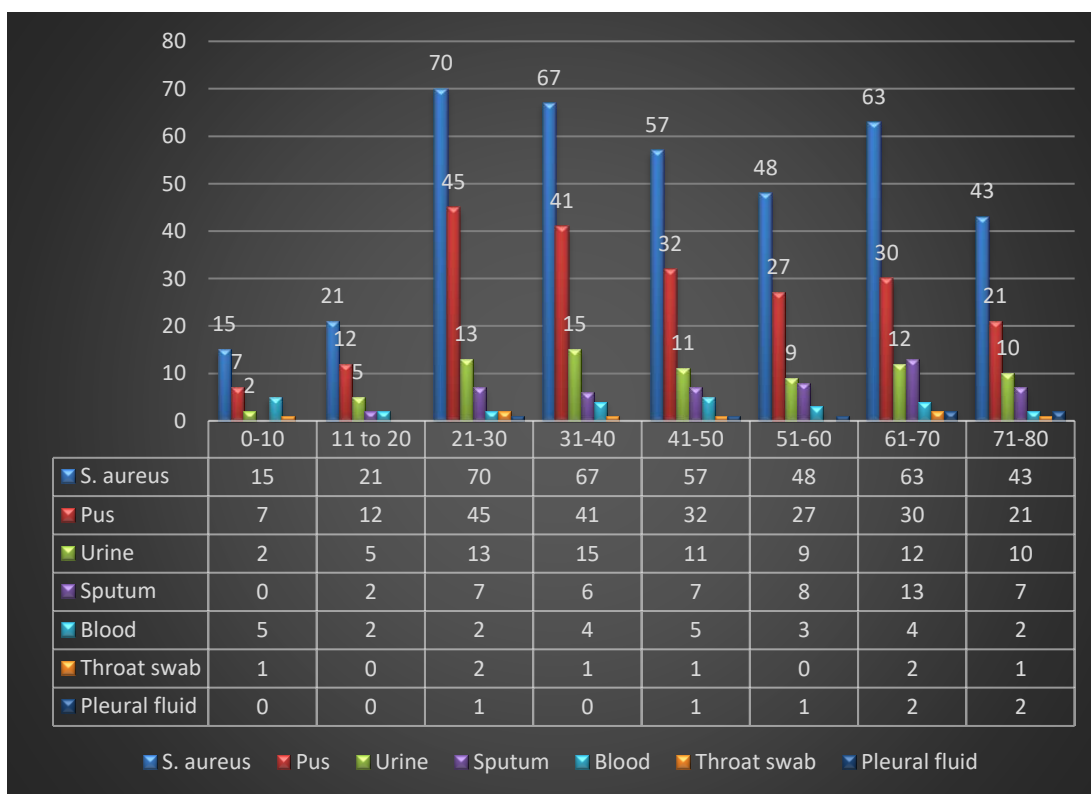
Among 215/384 (55.99%) *S. aureus* isolated from pus sample, maximum 45 isolates were from 21-30 age group, followed by 41 isolates were from 31-40 age group, 32 isolates were from 41-50 age group, 30 isolates were from 61-70 age group, 27 isolates were from 51-60 age group, 21 isolates were from 71-80 age group, 12 isolates were from 11-20 age group and 7 isolates were from 0-10 age group.

Among 77/384 (20.05%) *S. aureus* isolated from urine sample, maximum 15 isolates were from 31-40 age group, followed by 13 isolates were from 21-30 age group, 12 isolates were from 61-70 age group, 11 isolates were from 41-50 age group, 10 isolates were from 71-80 age group, 09 isolates were from 51-60 age group, 05 isolates were from 11-20 age group and 02 isolates were from 0-10 age group.

Among 50/384 (13.02%) *S. aureus* isolated from sputum sample, maximum 13 isolates were from 61-70 age group, followed by 08 isolates were from 51-60 age group, 07, 07, 07 isolates were from 21-30, 41-50, 71-80 age group, 06 isolates were from 31-40 age group and 02 isolates were from 11-20 age group.

Among 27/384 (7.03%) *S. aureus* isolated from blood sample, maximum 05, 05 isolates were from 0-10 and 41-50 age group, followed by 04, 04 isolates were from 31-40 and 61-70 age group, 03 isolates were from 51-60 age group, 02, 02, 02 isolates were from 11-20, 21-30 and 71-80 age group. Among 08/384 (2.08%) *S. aureus* isolated from throat swab sample, maximum 02, 02 isolates were from 21-30 and 61-70 age group, followed by 01, 01, 01, 01 isolates were from 0-10, 31-40, 41-50 and 71-80 age group.

Among 07/384 (1.82%) *S. aureus* isolated from pleural fluid sample, maximum 02, 02 isolates were from 61-70 and 71-80 age group, followed by 01, 01, 01 isolates were from 21-30, 41-50 and 51-60 age group.

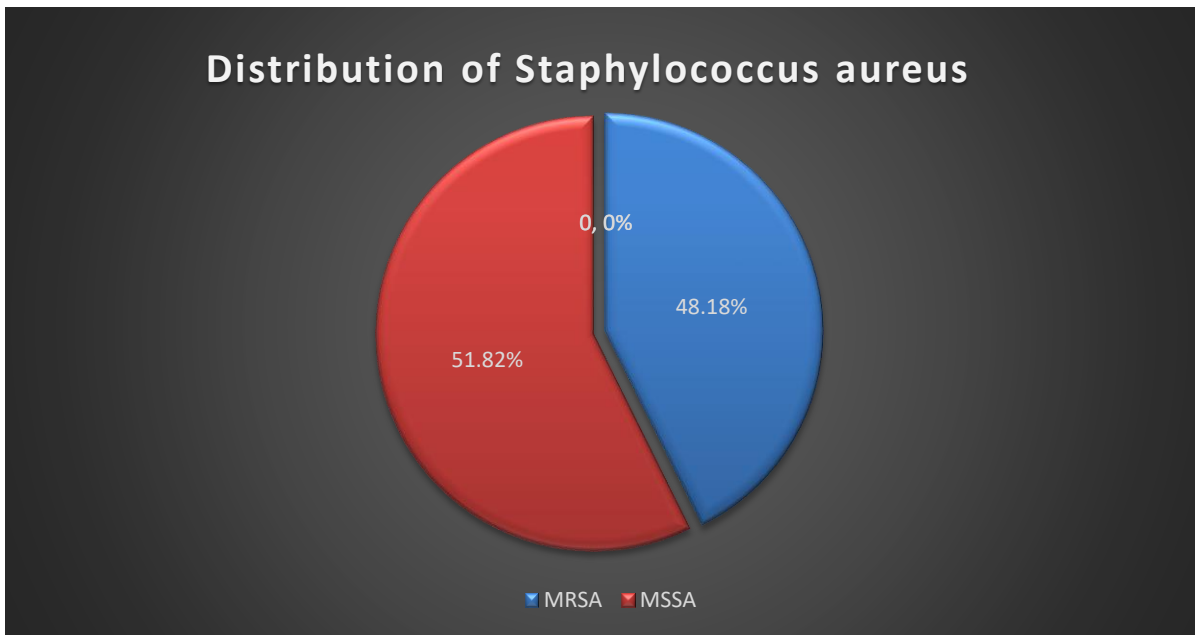


Graph No.5: Graphical Representation of Age group and Sample wise distribution of *S. aureus* isolates.

Table No.6: Distribution of *S. aureus* among MRSA and MSSA.

Staphylococcus aureus	384	100%
MRSA	185	48.18%
MSSA	199	51.82%

Among 384 *staphylococcus aureus*, 185 (48.18%) were methicillin resistant and 199 (51.82%) were methicillin sensitive.



Graph No. 6: Distribution of *Staphylococcal aureus* among MRSA and MSSA.

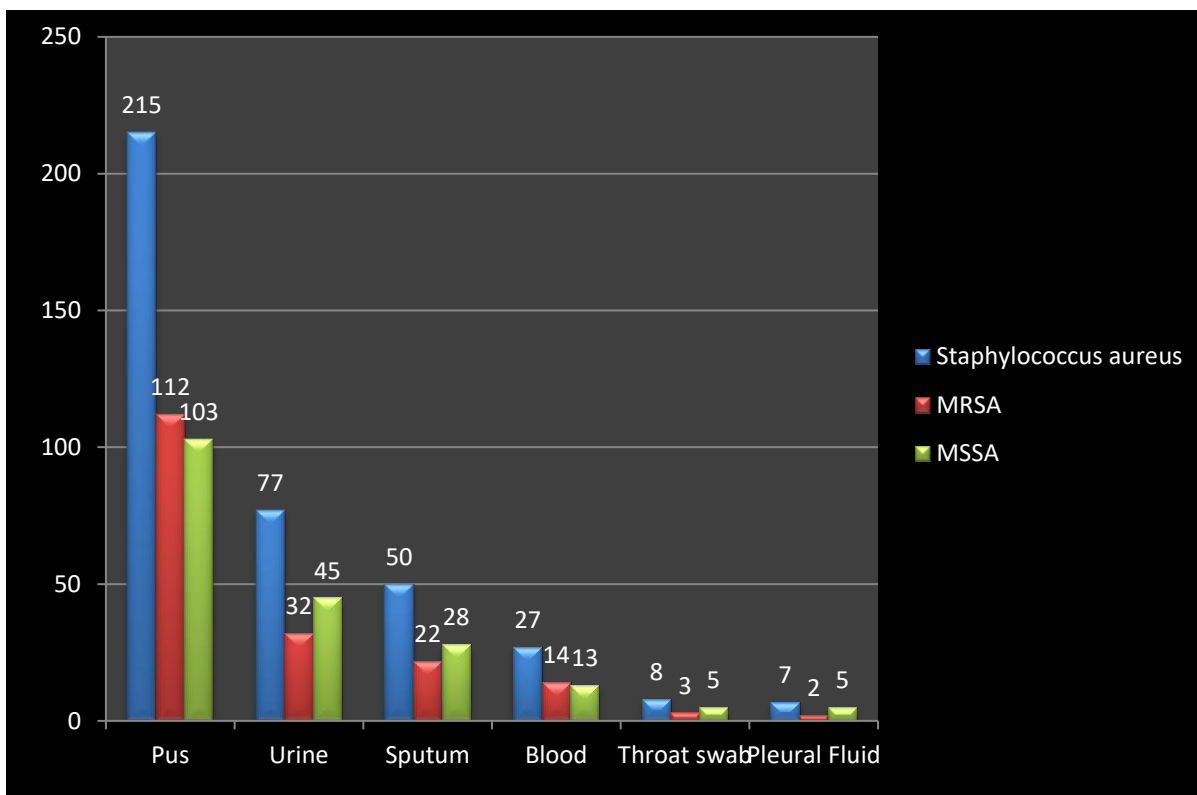
Table No. 7: Isolation of MRSA from different clinical specimens.

Sr. No.	Clinical Samples	<i>S. aureus</i> No. (%)	MRSA No. (%)	MSSA No. (%)
1	Pus	215 (55.99)	112 (52.09)	103 (47.91)
2	Urine	77 (20.05)	32 (41.56)	45 (58.44)
3	Sputum	50 (13.02)	22 (44)	28 (56)
4	Blood	27 (7.03)	14 (51.85)	13 (48.15)
5	Throat swab	08 (2.08)	03 (37.5)	05(62.5)
6	Pleural Fluid	07 (1.82)	02 (28.57)	05 (71.43)
Total		384 (100)	185 (48.18%)	199 (51.82%)
df		5		
χ² test		5.073		
P value		0.407^{NS}		

χ² : Chi square test df: Degree of freedom NS: Non-significant (p>0.05 at 95% CI(ConfidenceInterval))

Among 185 (48.18%) MRSA isolates highest number was obtained from pus112, followed by urine32, sputum 22, blood14,throat swab3 and pleural fluid 2.

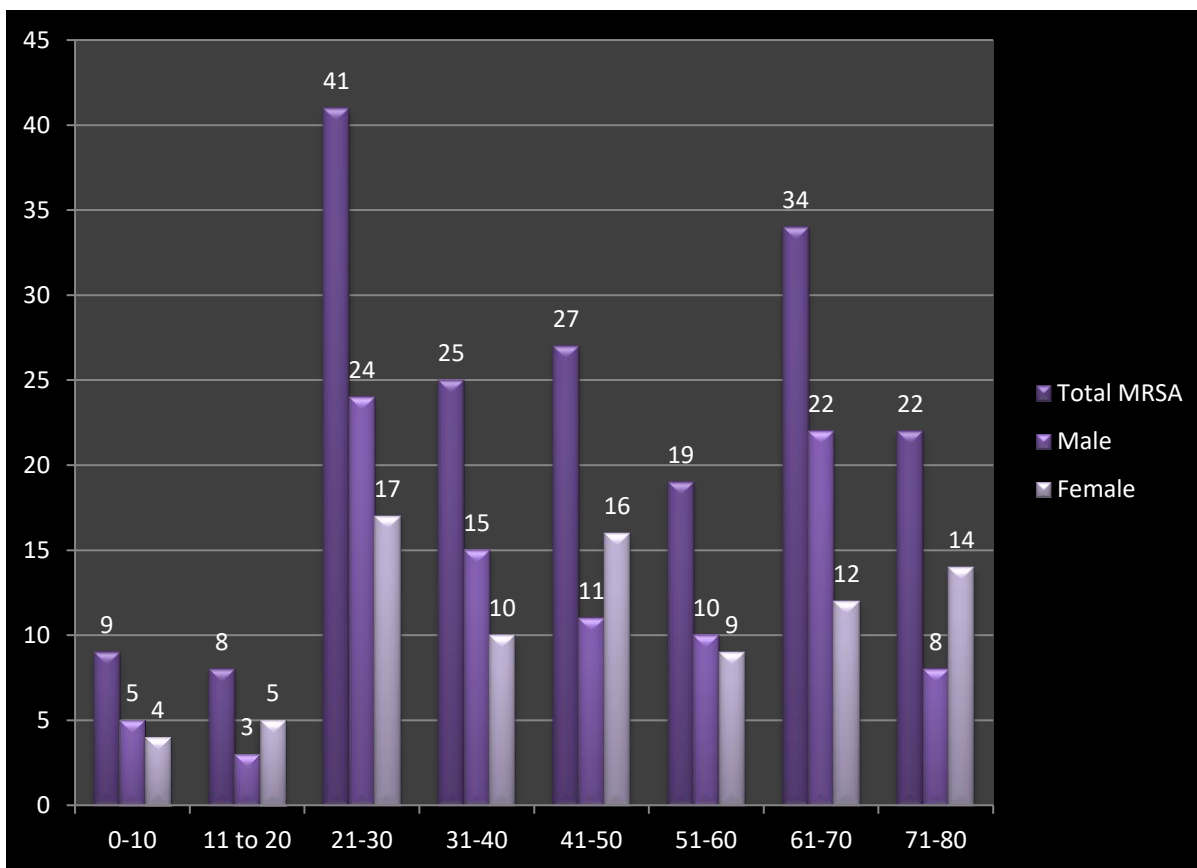
Among 199 (51.82%) MSSA isolates highest number was obtained from pus 103, followed by urine 45, sputum 28, blood 13, throat swab 5 and pleural fluid 5. However, the result was insignificant statistically ($p > 0.05$).



Graph No. 7: Isolation of MRSA from different clinical specimens.

Table No.8: Age and sex wise distribution of MRSA isolates.

Sr. No.	Age group	MRSA No. (%)	Male No. (%)	Female No. (%)
1	0-10	9 (4.86)	5 (55.56)	4 (44.44)
2	11-20	8 (4.32)	3 (37.50)	5 (62.50)
3	21-30	41 (22.16)	24 (58.54)	17 (41.46)
4	31-40	25 (13.51)	15 (60)	10 (40)
5	41-50	27 (14.59)	11 (40.74)	16 (59.26)
6	51-60	19 (10.27)	10 (52.63)	9 (47.37)
7	61-70	34 (18.38)	22 (64.71)	12 (35.29)
8	71-80	22 (11.89)	8 (36.36)	14 (63.64)
	Total	185 (100)	98 (52.97)	87 (47.03)



Graph No.8: Age and Sex wise separation of MRSA isolates.

Table No. 9: Age and MRSA isolates

Age	<i>S. aureus</i> No. (%)	MRSA No. (%)	MSSA No. (%)
0-10	15 (3.9)	9 (4.86)	6 (3.01)
11-20	21 (5.46)	8 (4.32)	13 (6.53)
21-30	70 (19.77)	41 (22.16)	29 (14.57)
31-40	67 (17.44)	25 (13.51)	42 (21.11)
41-50	57 (14.84)	27 (14.59)	30 (15.07)
51-60	48 (12.5)	19 (10.27)	29 (14.52)
61-70	63 (16.4)	34 (18.37)	29 (14.57)
71-80	43 (11.19)	22 (11.89)	21 (10.55)
Total	384 (100)	185 (100)	199 (100)
df	7		
χ² test	11.76		
P value	0.108 ^{NS}		

χ² : Chi square test df: Degree of freedom NS: non-significant (p>0.05 at 95% CI(ConfidenceInterval))

Table No. 10: Gender and MRSA isolates

Gender	<i>S. aureus</i> No. (%)	MRSA No. (%)	MSSA No. (%)
Male	195 (50.78)	98 (52.79)	97 (48.74)
Female	189 (49.22)	87 (47.02)	102 (51.26)
Total	384 (100)	185 (100)	199 (100)

df	7
χ^2 test	11.76
P value	0.108 ^{NS}

χ^2 : Chi square test df: Degree of freedom NS: non-significant ($p > 0.05$ at 95% CI(ConfidenceInterval))

Among 185 MRSA isolates, 98 (52.97%) isolates belonged to male patients and 87 (47.03%) were from female patients. Among 98 MRSA isolated from male patients, 24 belonged to (21-30) age group followed by 22 from (61-70) age group, 15 from (31-40) age group, 11 from (41-50) age group, 10 from (51-60) age group, 8 from (71-80) age group, 5 from (0-10) age group, 3 from (11-20) age group.

Among 87 MRSA isolated from female patients, 17 belonged to (21-30) age group followed by 16 from (41-50) age group, 14 from (71-80) age group, 12 from (61-70) age group, 10 from (31-40) age group, 9 from (51-60) age group, 5 from (11-20) age group, 4 from (0-10) age group. Maximum MRSA isolates were observed in male patients. The results were insignificant statistically ($p > 0.05$)

DISCUSSION

Staphylococcal infections are a global health concern due to the increased medication resistance produced by these organisms. It accounts for 30% of hospital-acquired infections, with Staphylococcus being the most often isolated organism on culture in around 50% of bloodstream infections. *Staphylococcus aureus* poses a public health problem due to its rising virulence and resistance patterns, which concerns WHO. Increasing antibiotic resistance is a worrisome trend being observed worldwide. Methicillin-resistant *Staphylococcus aureus* (MRSA) was first reported in the United Kingdom (UK) in 1961 and has since been reported throughout the world as a nosocomial pathogen [16]. In recent years, vancomycin has been the preferred treatment for MRSA (Methicillin Resistant Staphylococcus aureus) infections. However, it has led to the emergence of vancomycin intermediate Staphylococcus aureus (VISA) and vancomycin-resistant Staphylococcus aureus (VRSA).

A steady increase in the occurrence of MRSA strains has been reported in hospitals globally including in India. MRSA is a notorious pathogen that can withstand and develop resistance against the available empirical drugs of choice. The availability of sensitive and specific methods for the accurate detection of antibiotic resistance in these bacteria has become an important tool in clinical diagnosis [17-19].

In the present study the prevalence of MRSA was observed to be 48.1%. This study was in support with the study performed by the other authors Gadepalli R *et al.*, in 2009 and Gopalakrishnan R, *et al.*, in 2010 where the prevalence was found to be 41% and 40% respectively [20,21]. Another study in India [22] recorded the prevalence of MRSA as 40-50 per cent. but in contrast with the study by Maj Puneet Bhatt *et al* in 2015 where the prevalence rate was observed to be 20% [23].

Table No. 11: Comparison of age-based prevalence of *Staphylococcus aureus* isolates with other studies

Present study (N=384)	Kaur K <i>et al</i> (N=162) [24]	Goyal A <i>et al</i> (N=379) [25]	Mandal M <i>et al</i> (N=108) [26]
21-30 years (70/18.23%)	16-40 years (70/43.2%)	20-40 years (193.29/51%)	21-30 years (39/36.11%)
31-40 years (67/17.45%)	-	0-20 years (98.54/26%)	11-20 years (23/21.29%)

In this present study, there were total 384 isolates of *Staphylococcus aureus*, of which maximum isolates were obtained from the age group of 21-30 years (18.23%) followed by 31-40 years (17.45%). Our results were comparable to the study of Mandal M *et al* and Goyal A *et al* which showed high prevalence in the age groups of 21-30 years (36.11%) and 20-40 years (51%) respectively. However. In the study of Kaur K *et al* (table 11), the prevalence was high in the age group ranging from 16-40 years (43.2%). This could be due to wider margin of age range selected in their study.

Table No. 12: Comparison of gender based (Male: M, Female F) prevalence of *Staphylococcus aureus* isolate with other studies

Present Study (N=384)		Kaur K et al (N=162) [24]		Mandal M et al (N=108) [26]		Sarrafzadeh F et al (N=250) [27]		Adhikari R et al (N=95) [28]	
M	F	M	F	M	F	M	F	M	F
195 (50.78%)	185 (49.22%)	85 (52.4%)	44 (47.5%)	37 (34.26%)	71 (65.74%)	188 (75.2%)	62 (24.8%)	41 (43.16%)	54 (56.84%)
Ratio	1.03:1	1.93:1		0.52:1		3.03:1		0.76:1	

Of the total isolates, 195 *Staphylococcus aureus* (50.78%) isolates were from males while 189 isolates were from females (49.22%) with male:female ratio of 1.03:1. Among males, the maximum isolates were from the age group of 21-30 years while females it was 31-40 years (table 6.2).

In the study of Kaur K *et al*, there were 162 isolates of *Staphylococcus aureus*, of which 85 (52.4%) were obtained from females while 44 (47.5%) were obtained from male patients. The higher number of isolates was obtained from male patients which was similar to our study. The male:female ratio was 1.93: which was higher than our study. The findings of the present study were also similar to that of Sarrafzadeh *Fetal* who showed higher prevalence in males (188/75.2%) compared to females (62/24.8%). The male: female ratio was very high in comparison to our study (3.03:1). In contrast, a study from Bihar conducted by Mandal M *et al* and a study conducted in Nepal by Adhikari R *et al* reported high prevalence of *Staphylococcus aureus* isolates in females compared to males. In females it was 71 (65.74%) and in males it was 37 (34.26%) in study of Mandal M *et al* and 41 (43.16%) and 54 (56.84%) respectively in study of Adhikari R *et al*. The male to female ratios in their study was low. i.e. 0.52:1 and 0.76:1, which were less in comparison to this study.

Table No.13 : Comparison of prevalence of *Staphylococcus aureus* isolates with other studies according to IPD and OPD

Present Study (N=384)		Kaur K et al (N=162) [24]		Joshi S et al (N=12442) [29]		Maharjan M et al (N=74) [30]		Kaleem F et al (N=276) [31]	
IPD	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD	OPD
45 (11.75%)	339 (88.28%)	102 (62.96%)	60 (37.03%)	9084 (73.01%)	3358 (26.98%)	59 (79.73%)	15 (20.27%)	185 (67%)	91 (33%)

In this study, 339 (88.28%) of isolates were obtained from outpatients while 45 (11.75%) of isolates were from inpatients. In contrast to this study, the meta-analysis study of Joshi S *et al* reported majority of isolates to be from inpatients. The total number of isolates from inpatients was 9084 (73.01%). Their findings were similar to that of Kaur K *et al* and Maharjan M *et al* who also reported higher number of isolates from inpatients. The prevalences were 102 (62.96%) and 9084 (79.73%)

respectively (table 6.3).Likewise, another study of Kaleem F *et al* also reported high prevalence of Staphylococcus aureus isolates (MRSA) in inpatients (67%).

Table No. 14: Comparison of prevalence of *Staphylococcus aureus* isolates with other studies according to various clinical specimen

Present study (N=384)	Usha MG et al N=190 [32]	Kandel SN et al (N=39) [33]	Kaur K (N=162) [24]	Nepal N et al (N=80) [34]	Maharjan M et al (N=74) [30]
Pus 215 (56%)	Pus 126 (66.31%)	Urine 9 (23.1%)	Pus and Wound 96 (59.2%)	Pus 42 (52.5%)	Wound swab 27 (47.4%)
Urine 77 (20%)	Blood 16 (8.42%)	Pus 8 (20.5%)	Urine 30 (18.5%)	Wound swab 21 (26.3%)	Pus 16 (41%)

In the present study most of the *Staphylococcus aureus* isolates i.e.215 were obtained from pus samples that accounted for 56% followed by urinesamples, the incidenceof which were 77 (20%) (table 6.4). Like the present study, the number of *Staphylococcus Aureus* isolates was high is pus sample in the studies conducted by Usha MG *et al* (126/66.31%), Kaur K et al (96/59.2%) and Niranjana N *et al* (42/52.5%) followed by blood (16/8.42%), urine (30/18.5%) and pus (16/41%) respectively. In contrast to our study, the study of Kandel SN *et al* showed higher incidence in urine sample (9/23.1%) followed by pus (8/20.5%). Another, study of Maharjan M *et al* reported higher incidence in wound swab (27/47.4%) followed by pus sample (16/41%). In their study, 26.2% of isolates were from ICU while 20.8% were isolated from Gynae ward.

Staphylococcus aureus is mainly found in environment and the surface of skin which could be the probable reason from high incidence of isolates from wound and pus samples. The pus and wound swab also are the potent causes of skin or soft tissue infection and surgical site infections [34].

In the present study, it was found that, out of 195 isolates of *Staphylococcus aureus* isolated from male patients, 60.5% was obtained from pus followed by 17.9% from the sputum sample, 11.3% from urine, 6.14 from blood, 2.6% from throat swab and 1.5% from pleural fluid. In case of 189 isolates obtained from female patients, 51.3% were obtained from pus sample, 29.1% from urine, 7.9% each from blood and sputum, 2% from pleural fluid and 1.6% from throat swab. With respect to age wise segregation of *Staphylococcus aureus* isolates from different clinical samples, we found that higher incidences were observed in the pus samples obtained from the patients belonging to 21-30 years of age. In case of isolates from urine sample maximum isolates were in the age group of 31-40 years.

Table No. 15: Comparison of prevalence of MRSA among *Staphylococcus aureus* isolates with other studies

Present Study (N=384)		Osman MM et al N=61 [35]		Arora S et al N=250 [36]		Jayshree et al N=29 [37]		Thati V et al N=358 [38]	
MSSA	MRSA	MSSA	MRSA	MSSA	MRSA	MSSA	MRSA	MSSA	MRSA
199 (51.82%)	185 (48.18%)	36 (59%)	25 (41%)	135 (58.7%)	115 (41.3%)	17 (58.62%)	12 (41.38%)	73 (20.4%)	285 (79.6%)

The present study reported that, of 384 *Staphylococcus aureus* isolates, 185 (48.18%) were methicillin resistant (i.e. MRSA) while 199 (51.82%) were methicillin sensitive (MSSA). In the study of Osman MM *et al*,25 (41%) of *Staphylococcus aureus* isolates were MRSA while 36 (59%) were MSSA which was similar to our study. Likewise, in the study of Jayshree N et al, the prevalence of MRSA was 41.38% (12) while the study of Thati V *et al* showed a very high prevalence of MRSA i.e. 79.6% (285) which was higher than that observed in our study (table 15).

Table No.16: Comparison of prevalence of MRSA isolates in various clinical specimen with other studies

Present study (N=185)	Kandel SNet <i>al</i> (N=18) [33]	Kaleem Fet <i>al</i> (N=276) [31]	Rana Khara R <i>et al</i> (N=52) [39]	Joshi S <i>et al</i> (N=2695) [29]
Pus (112/60.54%)	Wound swab (7/38.8%)	Pus (190/69%)	Blood (19/36.54%)	Pus (1717/63.71%)
Urine (32/17.29%)	Pus (4/22.2%)	Respiratory sample (20/18%)	Pus (15/28.85%)	Blood (483/17.92%)

In this study, MRSA isolates was more in pus samples, i. e, 112 which accounted for 60.54%. Similar was the case for MSSA isolates. More isolates were obtained from pus sample (32/17.29%). There was no significant association between and the clinical samples and the incidence of MRSA and MSSA isolates. The result of our study was supported by the previous studies of Kaleem F *et al* and Joshi S *et al* who also reported higher prevalence of MRSA isolates in pus sample (69% and 63.71% respectively). However, in the study of Kandel SN *et al* more number of MRSA isolates were obtained wound swab (38.8%) while Rana Khara *et al* reported more prevalence of MRSA in blood samples (36.54%). These reports were in contrast to that of our study (table 16).

Likewise, a study from Chennai reported 40-50% incidence of MRSA strain [22] of which 17% was related to catheter related blood stream infections.

Table No. 17: Comparison of age-based prevalence of MRSA isolates with other studies.

Present study (N=185)	Kaur K <i>et al</i> (N=83) [24]	Sarrafzadeh F <i>et al</i> (N=250) [27]	Lama U <i>et al</i> N=57 [40]
21-30 years (41/22.16%)	16-40 years (36/43.2%)	19-64 years (173/69.2%)	21-30 (12/17.9%)

In this study, the prevalence of MRSA isolates was more in the age group of 21-30 years (41/22.16%). It was in support of the study of Lama U *et al* who also reported high prevalence in 21-30 years age group (12/17.9%). In contrast to the present study, Kaur K *et al* and Sarrafzadeh F *et al* reported high prevalence in the age groups of 16-40 years (43.2%) and 19-64 years (69.2%) respectively (table 17).

Table No. 18: Comparison of gender-based prevalence of MRSA isolate with other studies

Present Study (N=185)		Rana Khara <i>et al</i> (52) [39]		Lama U <i>et al</i> (57) [40]	
Male	Female	Male	Female	Male	Female
98 (52.97%)	87 (47.03%)	32 (61.54%)	20 (38.46%)	27 (47.37%)	30 (52.63%)

The present study documented a greater number of MRSA isolates in male patients(98/52.97%) compared to female patients (87/47.03%). Similar to this study, study of Rana Khara *et al* more prevalence in male patients (32/61.54%). However, in contrast to this study, study of Lama U *et al* documented more prevalence in female patients (30/52.63%) (table 18).

Table No.19: Antimicrobial susceptibility pattern among MRSA and MSSA isolates.

STU DY	Y E A R	AK MRS A/M SSA	COT MRS A/M SSA	CL MRS A/M SSA	CD MRS A/M SSA	E MRS A/M SSA	LE MRS A/M SSA	TE MRS A/M SSA	TEI MRS A/M SSA	LZ MRS A/M SSA	VA MRS A/M SSA	NIT MRS A/M SSA
Prese nt study	20 22	76.22 %/81. 9%	25.95 %/54. 77%	44.32 %/71. 86%	63.78 %/67. 83%	56.22 %/60. 3%	66.49 %/69. 84%	72.97 %/82. 91%	89.73 %/93. 46%	96.22 %/97. 99%	97.3 %/10 0%	75%/ 91.11 %
<i>Moh anty S et al [41]</i>	20 19						62.99 %/84. 71%				100 %/10 0%	100 %/10 0%
<i>Lam a U et al [42]</i>	20 17	85.96 %/10 0%	-	5.26 %/90 %				43.85 %/10 0%				
<i>Ghos h S et al [43]</i>	20 16	-	45.45 %/40 %	-	45.45 %/62. 85%	18.18 %/37. 14%				100% /100 %	100 %/10 0%	
<i>Rana Khar a et al [44]</i>	20 16				40.38 %/77. 08%	36.54 %/77. 08%	86.54 %/10 0%			96.15 %/10 0%	100 %/10 0%	
<i>Jyoti K et al [45]</i>	20 16							37.5 %/18. 22%	100% /100 %			
<i>Osm an MM et al [46]</i>	20 16							64%/ 25%				
<i>Dha nala kshm i TA et al[47]</i>	20 12	-	67.86 %/68. 8%	-								
<i>Shitt u O et al [48]</i>	20 11							0/52. 63%	100% /100 %	100% /100 %		
<i>Aror a S et al</i>	20 10	62.6 %/91. 11%	-	-						98.26 %/10 0%	100 %/10 0%	46.15 %/50 %

[49]												
Taj V et al [50]	20 10	82.76 %/93. 84%	-	-								
Path ak A et al [51]	20 10	81.25 %/90. 24%	25%/ 46.34 %	-	56.25 %/95. 12%	50%/ 81.71 %	62.5 %/74. 39%		100% /100 %			

From the table no. 19 it was observed the comparison of the sensitivity pattern evaluated in different research. It was also noted that Vancomycin was found to be the most sensitive with the sensitivity of 97.3%, followed by Linezolid 96.2% and then Teicoplanin 89.73 % was found to be the most effective drug of choice. The Co-trimoxazole and Chloramphenicol were found resistance with 25.95% and 44.32% respectively.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become commonplace globally since it was initially recognised in a UK hospital in 1961, and poses a global threat causing serious infections in health facilities and the community, contributing 60% more deaths than the non-resistant form of the infections [52]. Recent developments in new antimicrobials against MRSA include ceftaroline, ceftobiprole, dalbavancin, oritavancin, iclaprim and delafloxacin, all of which are in various phases of clinical studies. Despite ongoing development of new treatments, active surveillance efforts, and advancements in infection control, the incidence of MRSA has emerged as a major source of morbidity, higher costs of healthcare delivery services, and increased mortality in hospitals over the past decades [53,54].

CONCLUSION

Drugs used to treat MRSA infections should have both effective antibacterial activity and be cost effective. Oral dosage options for linezolid and chloramphenicol can allow hospitalised patients to be discharged early while reducing the likelihood of VRSA emergence. Good hospital infection control procedures prove to be the mainstay against these infections, because medications can never be an adequate substitute for good medical practice.

Declarations:

Conflicts of interest: There is no any conflict of interest associated with this study

Consent to participate: We have consent to participate.

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