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Research Article

**PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY
PATTERN OF METHICILLIN-RESISTANT
STAPHYLOCOCCUS AUREUS STRAINS ISOLATED FROM
DIFFERENT CLINICAL SPECIMENS AT KHYBER
TEACHING HOSPITAL, PESHAWAR, PAKISTAN**¹Zubair Hussain, ²Aman Ullah, ³Muhammad Asif Azeb, ⁴Noor Zada, ⁵Falak Niaz,
⁶Faiz Ullah^{1,2,6}Institute of Paramedical Sciences, Khyber Medical University Peshawar, Pakistan^{3,5}Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan⁴University of Peshawar, Pakistan**Abstract:**

OBJECTIVES: The aim of this study was to determine the prevalence and antimicrobial susceptibility pattern of Methicillin-Resistant *S. Aureus* (MRSA) strains isolated from different clinical specimens referred for routine culture and sensitivity testing. **METHODOLOGY:** A cross sectional study was conducted in which 642 consecutive clinical isolates of staphylococcus aureus were collected from various clinical specimens at Khyber Teaching Hospital Peshawar from January 2017 to September 2017. All the isolates were identified as per Clinical and Laboratory Standards Institute (CLSI) guideline. *S.aureus* isolates then were screened for MRSA by using Cefoxitin (30ug) disc. The antimicrobial susceptibility patterns of MRSA to ten commonly used antibiotics were determined by Kirby-Bauer disc diffusion method. Data were coded, entered and analyzed using SPSS version 22 and MS. Excel. **RESULTS:** A total of 642 clinical of *Staphylococcus aureus* were studied, out of which 148 (23.05%) were found positive for MRSA, among which the highest number was recovered from wound pus specimens 93 (62.4%) and least number was collected from blood 1 (0.7%). The frequency of MRSA was more in female 82 (55.4%) than in male 66 (44.59%). Out of these total MRSA, the greatest percentage were recovered from the patient of OPD 39.6%, and least percentage of MRSA isolates were recovered from ENT Ward and Burn Unit (0.7%). These MRSA strains also showed various degrees of resistance to Ciprofloxacin (84.45%), Trimethoprim-sulfamethoxazole (77.02%), Erythromycin (69.59%), Gentamicin (64.86%), Amikacin (58.10%), Clindamycin (34.45%), Doxycycline (31.75%), Linezolid (10.13%) and minimum resistant were found against Vancomycin (5.4%). **CONCLUSION:** In the present study, the prevalence of MRSA infection in KTH, Peshawar was 23.05%. Pus/abscess is the main source of MRSA than other samples in hospital settings. Vancomycin, Linezolid and Doxycycline showed best chemotherapeutic activity against MRSA infections.

KEY WORDS: *Staphylococcus aureus*; MRSA; Prevalence; Antibiotic susceptibility

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INTRODUCTION:

Staphylococcus aureus was discovered by a surgeon namely Alexander Ogston from a knee surgical abscess in the year 1880 (1). *Staphylococci* are Gram-Positive Cocci which are arranged in grape-like cluster. They produce golden yellow creamy or infrequently white colonies on Blood agar (BA), Chocolate agar (CA) having diameter about 1–2 mm following overnight incubation (2). When they seeded on blood agar aerobically then some strains show beta haemolytic appearance. They are non-spores forming bacteria but is resistant to high salt concentration and dry condition (2). The range of temperature for its growth is usually (10–40) °C, but optimum temperature is 35–37 °C (3).

In early 1940s, an aureus infection was fatal disease with the mortality rate for bacteraemia of about 80% (4). Penicillin was the most effective antibiotics to treat *S.aureus* infections since 1940s (5). However the first case of penicillin resistant *S.aureus* strains was reported in 1944s (5). This strain was called Penicillin-resistant *S.aureus* which is caused by acquisition of a plasmid encoding the penicillin-degrading enzyme penicillinase which degrades the penicillin (6). After the emergence of penicillin-resistant *S.aureus*, penicillinase-stable penicillin such as methicillin was introduced in 1959s for the treatment of infections caused by penicillin-resistant *S.aureus* (5). However, only two years later, the case of Methicillin-resistant *S.aureus* (MRSA) was reported in 1961 in England (3).

MRSA is a multi-drugs resistant pathogen which can cause life threatening sepsis, endocarditis, osteomyelitis, pneumonia, surgical site infections, toxic shock syndrome, septicaemia, scalded skin syndrome, etc (7). It can also easily colonize in immune-deficient patients and cause a variety of serious infections (8).

Annually, nosocomial infections accounts for morbidity and mortality among millions of patients, worldwide (4). The National Nosocomial Infections Surveillance System (NNISS) reported an increase of MRSA in large in United States hospitals from 4% in the 1980s to 50% in the late 1990s. In some hospitals, MRSA represented up to 80% of all *S.aureus* (9). The United States Centre for Disease Control and Prevention (CDC) estimates that about 12% of MRSA infections are now community-associated. MRSA alone cause more deaths in the United States in 2005 than human immunodeficiency virus infection. Sweden shows that only 2.1% isolates and Netherland that less than 1% isolates were MRSA (3). In India, the prevalence of MRSA ranges from 27% in Bombay to 47% in Delhi (10). In different areas of Pakistan, the reported studies up to the year 1999 show comparatively low prevalence (5.0-22.3%) of

MRSA. After that period, there is a progressive increase in MRSA prevalence (11). The frequency of MRSA has been observed by Hafeez et al (12) in the major cities of Pakistan and was found 42%. Another study was conducted from August 2012 to March 2013, a total of 855 clinical samples were studied, out of which 45 (5.26%) were found positive for MRSA, among which 69% were from males and 31% from female (13).

The overall aim of this study was to determine the Prevalence, antibiotic susceptibility pattern of Methicillin-Resistant *S. Aureus* (MRSA) and current status of MRSA in Khyber Teaching Hospital, MTI, Peshawar, Pakistan.

METHODOLOGY:

A cross-sectional study was conducted at Khyber Teaching Hospital (KTH) Peshawar from January 2017 to September 2017 to determine the prevalence and Antibiotic resistant pattern of Methicillin-Resistant *S. Aureus* (MRSA). The source population was the different clinical samples submitted to Microbiology Department of KTH for culture and sensitivity. That was collected from all inpatients and outpatients who came to the hospital for medical help during the study period. The sample size was determined using consecutive sampling technique which was limited by time from January 2017 to September 2017 therefore, a total of 642 *Staphylococcus aureus* strains were included among different clinical samples. .

Different clinical samples were collected from patients by employing standard microbiological procedures. Pus from wound, ear discharge, blood, nasal swab, throat swab, eye swab, vaginal discharge, urethral discharge, urine, sputum, and body fluids were clinical specimens collected. All specimens were transported to microbiology laboratory of the hospital with minimum delay for culture and sensitivity tests.

Clinical specimens were inoculated on to blood agar and mannitol salt agar by using streaking method. Inoculated plates were incubated at 35-37 °C for 18 to 24 hours aerobically. Bacterial colonies showing typical characteristics of *S. aureus* (i.e., beta hemolytic on blood agar and colonies with golden yellow pigmentation on mannitol salt agar) were subjected to subculture on to basic media, gram stain and biochemical tests like catalase and coagulase tests. Catalase positive and gram positive bacteria appearing in grape like cluster was spot inoculated to DNase agar. Inoculated DNase agar plates were incubated at 37 °C overnight and flooded with 1N HCl. Isolates that hydrolyzed DNA in DNase agar were considered *S. aureus*. Gram-positive Cocci identified on the basis of gram reaction, catalase, coagulase and DNase positive test results.

MRSA were screened by Kirby Bauer disc diffusion method by using Cefoxitin (FOX) (30 ug) disc on MHA plate. A suspension was prepared from each isolate which was equivalent to 0.5 McFarland standards. Then with the help of swab, lawn culture was made on Muller-Hinton agar plate incubated at 37 °C and after overnight incubation, zone of inhibition was measured. The interpretation was taken according to CLSI guidelines (14). If the diameter of inhibition zone was ≤ 21 mm then

RESULTS:

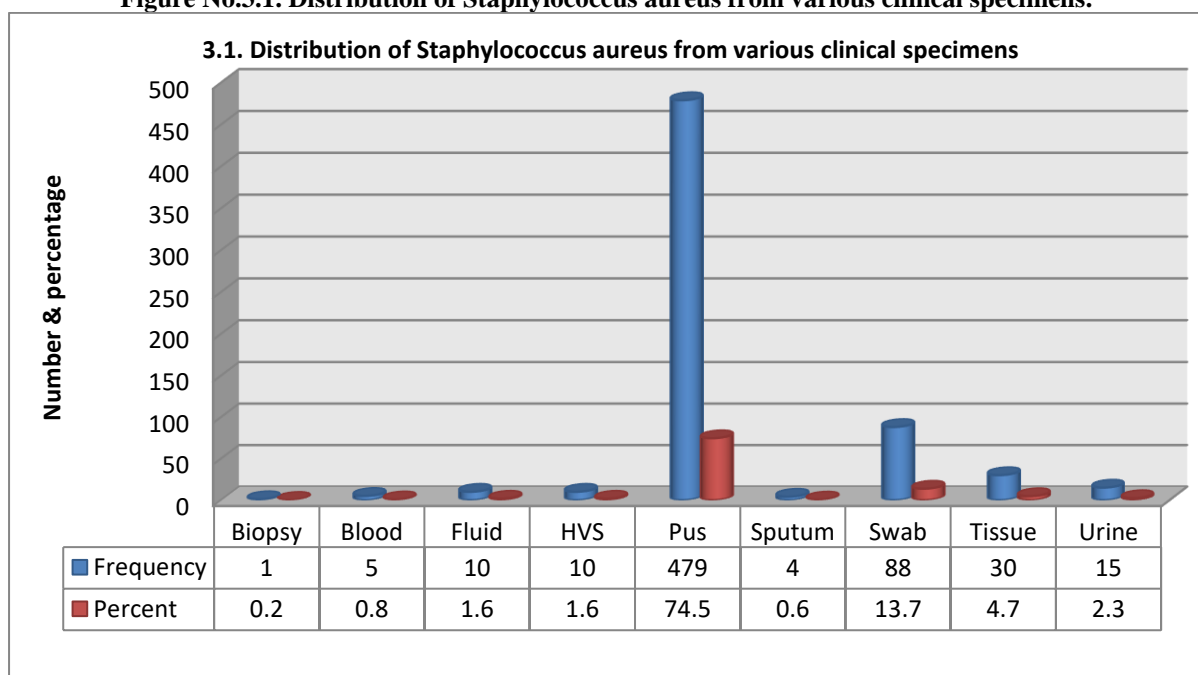
In the present study, we isolated a total of 642 *Staphylococcal aureus* strains from different clinical specimens collected from different patients. The various clinical samples from which *S. aureus* were isolated are shown in **Figure 3.1**. The highest

considered as Cefoxitin resistant (MRSA) and ≥ 21 mm was reported as Cefoxitin sensitive (MSSA).

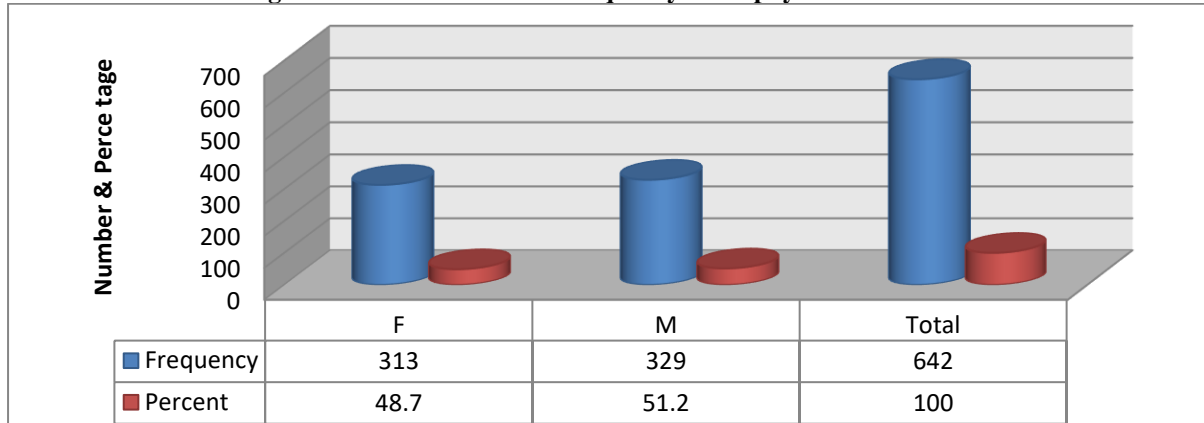
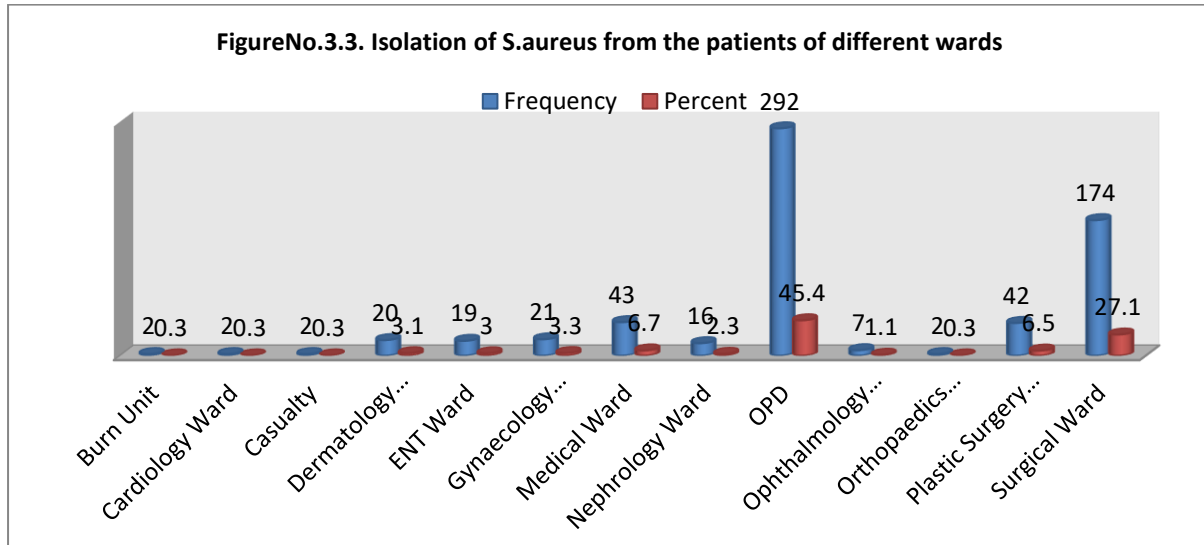
All data quality control tools of quality assurance that were incorporated in standard operating procedures (SOPs) of the microbiology laboratory were strictly followed. The data were checked for completeness and representativeness prior to entry. The reliability of the study findings were guaranteed by implementing quality control (QC) measures throughout the whole processes of the laboratory work.

percentage of these isolates were collected from pus (74.5%), followed by swab (13.7%), tissue (4.7%), Urine (2.3), body fluid (1.6%), High Vaginal Fluid (1.6%), blood (0.8%) and least number of isolates was recovered from sputum (0.6%) and biopsy (0.2%).

Figure No.3.1. Distribution of Staphylococcus aureus from various clinical specimens.



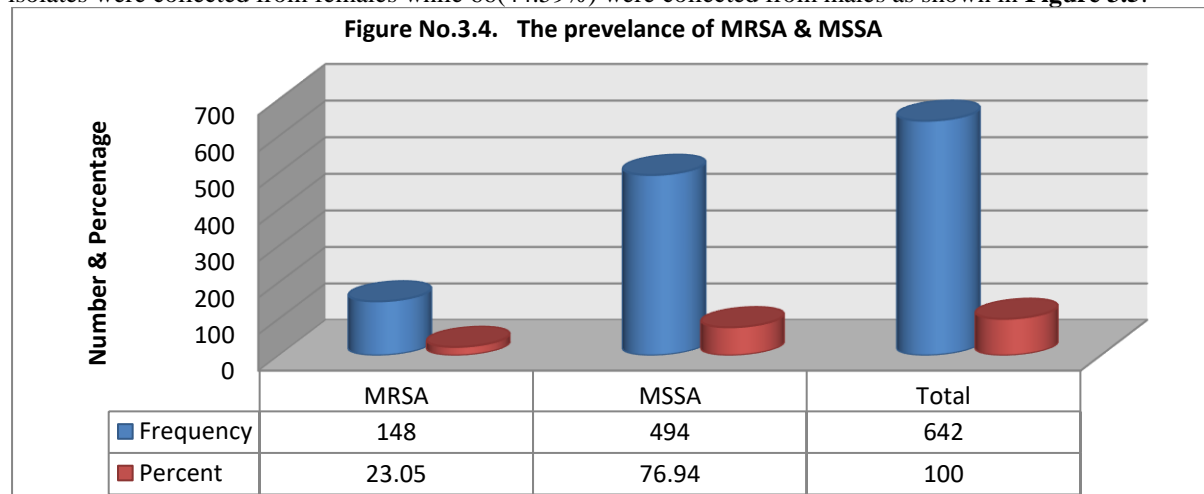
There were a total 642 *Staphylococcus aureus* case in our study, in which the frequency of *S. aureus* was more in male (51.2%) than female (48.7%) shown in **Figure 3.2**. These isolates were collected from different wards patients as well as from OPD and casualty patients are shown in **Figure 3.3**. Among all these isolates, the highest number of isolates was collected from OPD patients (45.4%) followed by Surgical ward (27.1%) and least number was recovered from casualty (0.3%), Burn Unit (0.3%) and Cardiology ward (0.3%).

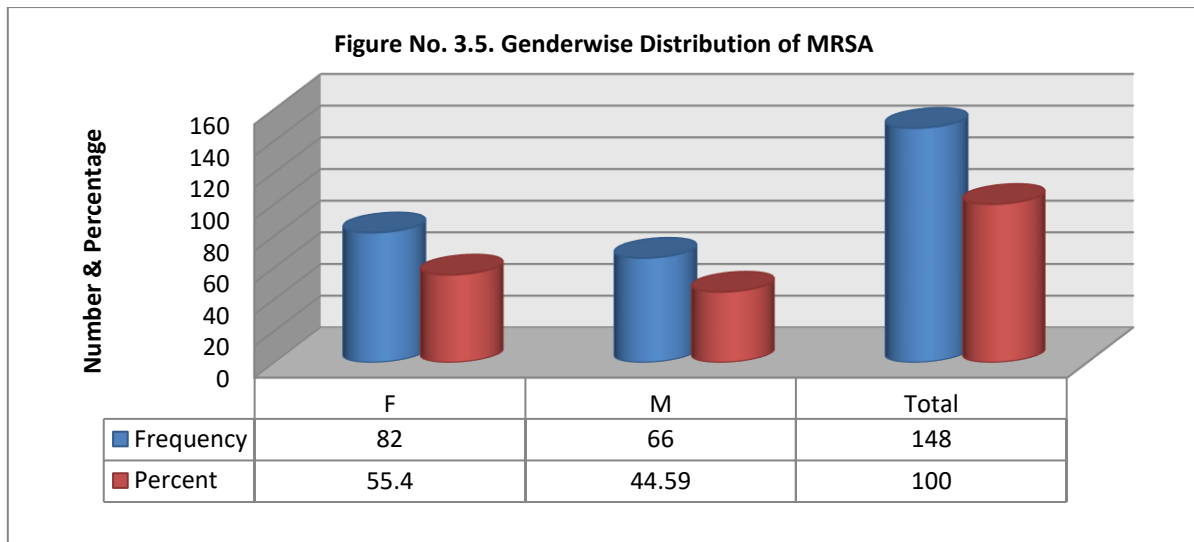
Figure No. 3.2. Gender wise frequency of *Staphylococcus aureus*FigureNo.3.3. Isolation of *S.aureus* from the patients of different wards

3.1. PREVALENCE OF MRSA

For the prevalence and antibiotics susceptibility pattern of MRSA, 642 different clinical samples of *S.aureus* were obtained from different clinical specimens and were processed for the isolation of MRSA during January 2017 to September 2017. Out of 642 isolates of *Staphylococcus aureus*, 148 (23.05%) were Methicillin-resistant *S.aureus* (MRSA) and 494 (76.94%) were MSSA as shown in **Figure 3.4**. Among these 148 MRSA isolates, 88 (55.4%) isolates were collected from females while 66(44.59%) were collected from males as shown in **Figure 3.5**.

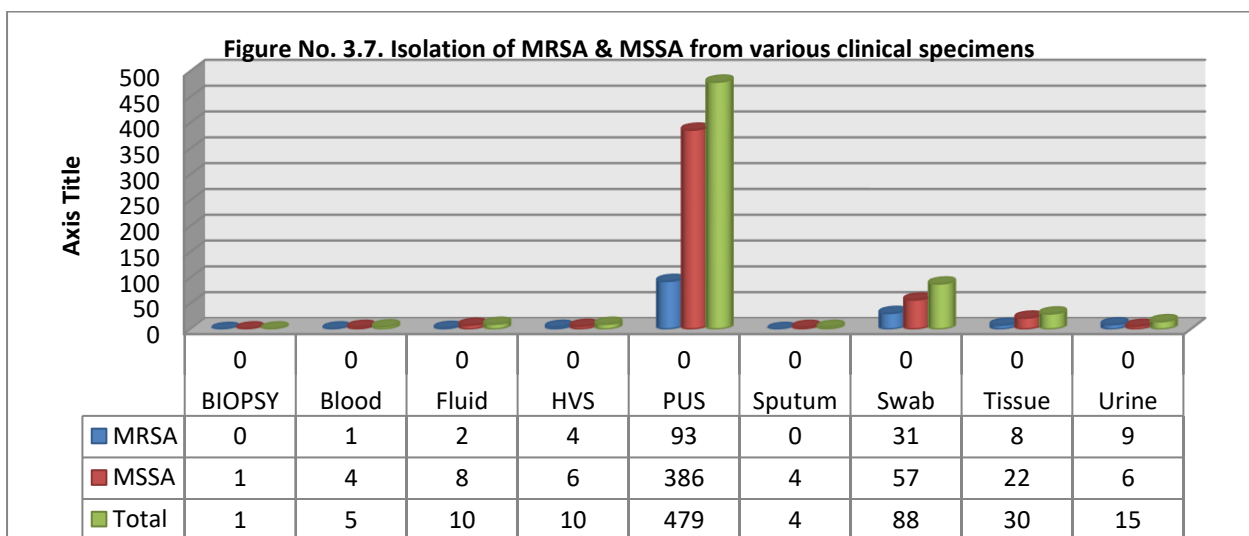
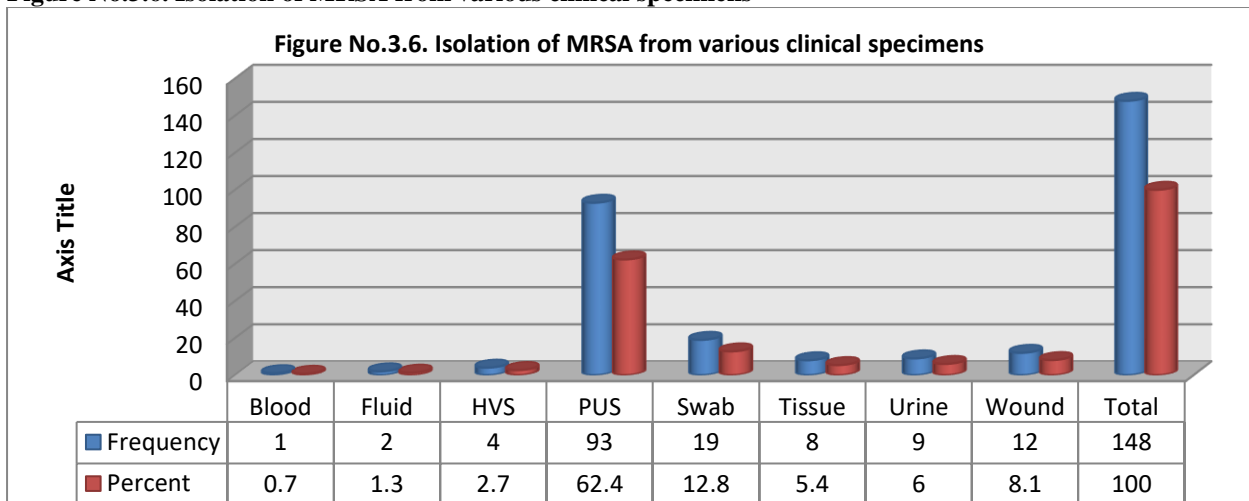
Figure No.3.4. The prevalence of MRSA & MSSA





The prevalence of MRSA was significantly variable among different clinical samples. Out of 148 isolates of Methicillin-resistant *Staphylococcus aureus* (MRSA), the highest number was recovered from pus 93 (62.4%), followed by swab 19 (12.8%), wound swab 12 (8.1%), urine 9 (6%), HVS 4 (2.7%), body fluid 2 (1.3%) and least number was collected from blood 1 (0.7%) as shown in **Figure 3.6 & 3.7**.

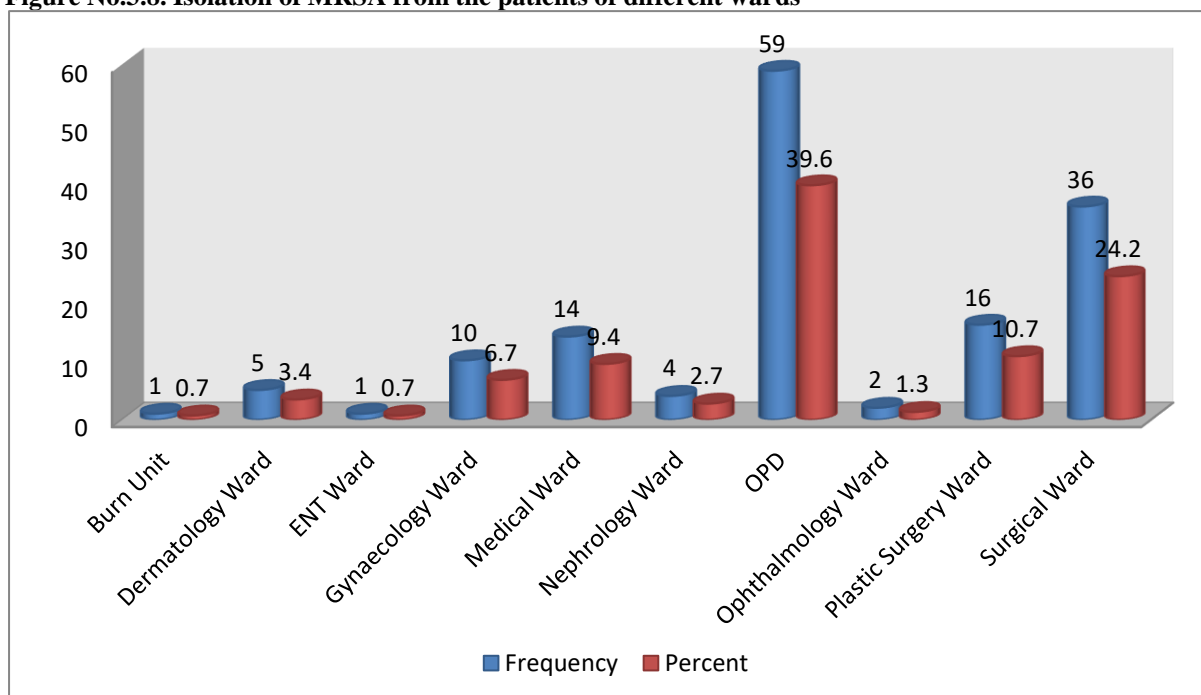
Figure No.3.6. Isolation of MRSA from various clinical specimens



The prevalence of methicillin-resistant *S.aureus* was also greatly variable among the patients of different wards. Out of 148 isolates of MRSA, the greatest percentage of MRSA isolates were collected from the patient of OPD 39.6%, followed by Surgical Ward 24.2%, Plastic surgery Ward 10.7%, Medical Ward 9.4%, Gynaecology Ward

6.7%, Dermatology Ward 3.4%, Nephrology Ward 2.7% and least percentage of MRSA isolates were recovered from ENT Ward 0.7% and Burn Unit 0.7% as shown in **Figure No.3.8**.

Figure No.3.8. Isolation of MRSA from the patients of different wards



3.2. ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF MRSA

The antibiotics susceptibility testing of all Methicillin-resistant *S.aureus* isolates was done against ten different of antibiotics by Kirby Bauer disc diffusion method as shown in **Table No.3.1**. The antibiotics resistant pattern of MRSA was significantly variable among different clinical samples. All the 148 MRSA isolates were found to be resistant to Ciprofloxacin (84.45%), followed by Trimethoprim-sulfamethoxazole (77.02%), Erythromycin (69.59%), Gentamicin (64.86%), Amikacin (58.10%), Fusidic Acid (40.54%), Clindamycin (34.45%), Doxycycline (31.75%), Linezolid (10.13%) and minimum resistant were found against Vancomycin (5.4%).

Table No.3.1. Antimicrobial susceptibility pattern of MRSA

Antibiotics	Concentration of antibiotics	Symbols	Resistance (Percentage)	Sensitive (Percentage)
Amikacin	[30 ug]	AK	58.1 %	41.8 %
Gentamicin	[10 ug]	CN	64.86 %	35.13 %
Fusidic Acid	[10 ug]	FD	40.54 %	59.45 %
Linezolid	[1-16 ug]	LZD	10.13%	89.86 %
Clindamycin	[30 ug]	DA	34.45%	65.54 %
Erythromycin	[15 ug]	ER	69.59 %	30.4%
Trimethoprim-sulfamethoxazole	[1.25/23.75]	SXT	77.02 %	22.97 %
Doxycycline	[30 ug]	DO	31.75 %	68.24 %
Ciprofloxacin	[5 ug]	CIP	84.45 %	15.54 %
Vancomycin	[30 ug]	VAN	5.4 %	94.59 %

DISCUSSION:

MRSA is not only confined to developed countries but an alarming increase in MRSA infection was also found in Pakistan in the last few decades. In the year 1987 (10), the first case of MRSA was observed

in Pakistan and after that a continuous increase in the prevalence of MRSA was reported (15) (16).

The prevalence of MRSA in the current study was found to be 23.05% which is lesser as compared to

the previous studies about MRSA in Pakistan and worldwide but found very high as compared to the previous one report Ahmad et al (5.26%) (17). The prevalence of MRSA in Peshawar, Pakistan is less than in South Africa, Cape Town (33—43%) (18), in eastern Uttar Pradesh, the big city of India (54.85%) (19), in California (86.3%) (20), 69.1% in western Nepal (21), (32%) in Kuwait (12), 71.4% in Sudan (22), 13% in Sri Lanka (17) and 33.5% in Russia (23). The overall prevalence of MRSA in Europe is 20% and in different European countries, the frequency of MRSA was reported as; Austria (8.8%), Belgium (23.6%), Bulgaria (33.9%), Croatia (36.7%), Czech Republic (5.9%), Denmark (0.6%), Estonia (0.9%), Finland (1%), France (33.1%), Germany (13.8%), Greece (44.4%), Hungary (7.1%), Iceland (0.5%), Ireland (41.2%), Israel (38.4%), Italy (40.9%), Luxemburg (19.2%), Malta (43.8%), Netherlands (0.6%), Poland (17.7%), Portugal (34.7%), Slovakia (10.5%), Slovenia (18.4%), Spain (24.8%), Sweden (0.8%), and United Kingdom (41.5%) (17).

The current study showed that the frequency of MRSA is higher in female 82/148 (55.4%) than in males 66/148 (44.59%). In 2012-13, a study was conducted (24), in which the frequency of MRSA were higher in male 69% than female (31%). In 2009-11, another study was conducted (13), in which they have found the frequency of MRSA greater in males (58%) than in females (42%) in Peshawar.

In the present study, the highest number of MRSA was recovered from pus 93 (62.4%), followed by swab 19 (12.8%), wound swab 12 (8.1%), urine 9 (6%), HVS 4 (2.7%), body fluid 2 (1.3%) and least number was collected from blood 1 (0.7%). In Peshawar a study was conducted by Ahmad et al., 2014 (17), they recovered the highest number of MRSA from pus samples (71%) and least number was recovered from blood (11%). Another similar study was conducted in Peshawar by Rahman et al (24), they have also recovered highest number of MRSA from pus (69.96%) least number was recovered from sputum (0.43%).

The antimicrobial susceptibility pattern of MRSA is very important in understanding new and emerging resistance trend as well as in the management of infections. The antimicrobial susceptibility pattern of all the 148 MRSA isolates was determined by disc diffusion method and as shown in **Table.1**. Here in KTH, Peshawar, MRSA show appreciable resistance to various antibiotics which is comparable to the other reports. In this study, Linezolid (89.86%), Doxycycline (68.24%) and Vancomycin (94.59%) showed fantabulous resistant against MRSA similar as reported in the previous reports of Pakistan (12)(13). All MRSA strains were resistant

to Cefoxitin (100%) show no therapeutic activity against MRSA infections.

Gentamycin was found to have 64.86 % resistance against MRSA and thus have poor therapeutic activity against MRSA infection. In previous reports of the different areas of Pakistan, gentamycin showed variable degree of resistant against MRSA; in Karachi (96.55%) (24), in Islamabad (100%), in Kohat 67% and in Rawalpindi 76% (17).

In the present study, all MRSA isolates shows greater resistance to Fusidic acid (40.54%), which is greater than in Karachi (2%) and in Islamabad (20%) (3). On the other hand, lower than the previous reports of Ahmad et al which was 66.7% in Peshawar (17). However, Fusidic acid is a drug of choice in most of the countries (25).

All 148 MRSA isolates showed 65.54% resistance against clindamycin. Higher resistance of MRSA against clindamycin has been reported in Karachi (90%) (13) and in Peshawar 71.11%. On the other hand, a lower resistance has been reported in United Kingdom (18%) (26), in Russia (27%) (8). In the present study, all MRSA strains also showed higher resistant against Ciprofloxacin (84.45%) and Trimethoprim-sulfamethoxazole (77.02%).

CONCLUSION:

In the present study, the prevalence of MRSA infection in KTH, Peshawar was 23.05%. Pus/abscess is the main source of MRSA than other samples in hospital settings. The frequency of MRSA was more in female 82 (55.4%) than in male 66 (44.59%) and the highest number of MRSA was recovered from the OPD and Surgical Ward samples. Vancomycin, Linezolid and Doxycycline showed best chemotherapeutic activity against MRSA infections.

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