



RESEARCH ARTICLE

Prevalence and Detection of *mecA* gene among Methicillin Resistant *Staphylococcus aureus* Isolates from Pus and Wound Samples of Indoor Patients in Kabul, Afghanistan

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ABSTRACT

Staphylococcus aureus is a non-sporulating gram-positive cocci which belongs to family Staphylococcaceae. It grows in grapelike structures when observed under microscope. *S. aureus* is the causative agent of various infections both in hospital and community settings. The pathogen offers a great public health threat due to its wide range of infections and its multi-drug resistance. Methicillin-resistant *S. aureus* is one of its most violent forms which show resistance to methicillin along with other beta lactam drugs. Methicillin resistant gene known as *mecA* gene expresses penicillin binding protein which makes the bacteria resistant against penicillin, methicillin, ceftioxin and many more antibiotics. In the present study, *S. aureus* isolates were collected followed by frequency determination of MRSA phenotypically using biochemical tests such as catalase, oxidase and coagulase tests. We determined MRSA by Kirby-Bauer disk diffusion method using cefoxitin and penicillin as standard antibiotics. The presence of *mecA* gene in MRSA isolates was determined by amplification of the gene by Polymerase Chain Reaction using specific primers and conditions. The results of the current study showed a high frequency of MRSA. A total of 150 isolates of *S. aureus* were collected and included in the study from two tertiary care hospitals and one diagnostic laboratory in Kabul city. A total of 100 out of 150 were phenotypically confirmed as MRSA. The frequency of MRSA infections was higher in females (77.33%) as compared to males (56%). The frequency of MRSA was higher in age group of elderly people of age 41 years to 75 years (79.03%) followed by age group of children from 1 year to 20 years (71.42%) and least in young adults of age group 21 years to 40 years (45.65%). Out of 100 confirmed MRSA isolates 86 isolates showed the presence of *mecA* gene. The study concluded that there is a high frequency of MRSA (66.6%) infection in the Kabul, Afghanistan. The aged people, children and females were more frequently infected with MRSA. The *mecA* gene was frequently found among most of MRSA isolates however, it is possibly associated with other genes and factors which need to be explored and studied. The study can further be extended in different ways like associating *mecA* genes with MSSA infections and determining the association of *mecA* genes with other factors associated with MRSA and multi-drug-resistant *S. aureus*.

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1. Introduction

Among pathogenic bacterial pathogens the *Staphylococcus aureus* is responsible for a number of infections ranging from very minor infections of skin like boils and pimples to

serious, impetigo to chronic invasive infections starting from carbuncles, abscesses, folliculitis cellulitis and life-threatening toxic shock syndrome [1, 2]. The emergence of

antimicrobial resistance (AMR) against various antibiotics in *S. aureus* especially resistant towards β -lactam antibiotics including methicillin known as MRSA (methicillin-resistant *S. aureus*) complicate the treatment due to the non-availability of specific antibiotics and hence the pose a serious challenge in management and complication of treatment of the infections [3].

Generally, there is a presence of SCCmec (staphylococcal cassette chromosome mec) which is called as mobile genetic elements including *mecA* gene or homologs *mecC* or *mecB* in MRSA isolates. Except for the antibiotic ceftaroline, the affinity towards other beta-lactam antibiotics has been minimized by production of a modified penicillin-binding protein (PBP2a) by the gene *mecA* [4]. Antibiotics resistance against various other antibiotics including macrolides, fluoroquinolones, and aminoglycosides is also commonly reported among MRSA isolates making them multi-drug resistant (MDR) [5]. Most commonly the MRSA have been found to colonize in the internal nose space making a potential reservoir for causing and transmission of infection [6]. Beyond the animate surfaces, the MRSA could also persist on inanimate surfaces for week to months and henceforth it is necessary to use decolonizing techniques to clean the environmental space and instruments to decrease the spread and prevent the infection [7].

The global burden of MRSA infections reaches up to fifty (50) million infections per year [8] as reported 14.58% prevalence by a comprehensive meta-meta-analysis all over the world [9]. Similarly, the enhancement in MRSA incidence and highest prevalence of 9.23% of MRSA from a study in South Asia making a serious issue for both healthcare and community settings [10]. Being the regional issue, the characterization and antimicrobial resistance of MRSA in Afghanistan is poorly reported. Likewise, the reports from neighbor's countries are with inconsistent prevalence ranging from 13% to 94%, mostly single center study and frequently fragmented [11, 12, 13].

The consistent ignorance, lack of clear and wide range of data on MRSA associated infections in Afghanistan create an important knowledge gap which is necessary for the development of evidence-based, efficient and national-wise prevention, control and therapeutic measures. The current study was a struggle for addressing the gap in data from various centers across Kabul province, Afghanistan. The aim of the study was to evaluate the prevalence and detection of *mecA* gene among Methicillin Resistant *Staphylococcus aureus* isolates from pus and wound samples of indoor patients in Kabul, Afghanistan.

2. Methodology

2.1 Study Design, Study Setting and Ethical Considerations

This experimental study was conducted in the Microbiology Research Laboratory (MRL), Department of

Microbiology and Medical Laboratory Technology, Faculty of Medicine, Afghanistan International Islamic University, Kabul, Afghanistan. Samples were collected from two tertiary care hospitals and one advanced diagnostic medical laboratory in Kabul city: Al-Hayat Hospital (AHH), Yakta Hospital, and Axon Bahar Medical Laboratory (ABML). A total of 150 pus and wound samples were included in the study, with 50 samples collected from each participating facility. The study population was further categorized according to sex and age. Participants were divided into three age groups: 1–20 years, 21–40 years, and 41–75 years. Prior to start, the ethical approval for this study was obtained by the Ethical Review Board of Axon Bahar Medical Diagnostic Laboratory, Kota e Sangi, Kabul, Afghanistan with approval No. Ref_AMD-L-RP-0001.

2.2 Sample Collection

A total of 150 pus and wound samples were collected from indoor patients at the participating healthcare facilities. Fifty samples were obtained from each of the three study sites. The sample size was determined using the World Health Organization sample size calculator based on the expected prevalence of infection in the study population. Demographic information, including sex and age, was recorded for the collected samples. The samples were transported to the laboratory for microbiological examination and isolation of *Staphylococcus aureus*.

2.3 Isolation and Identification of *Staphylococcus aureus*

The collected pus and wound samples were processed using standard microbiological techniques for isolation and identification of *S. aureus*. Identification was based on colony morphology, Gram staining, and biochemical characteristics. Following isolation, suspected *S. aureus* colonies were subjected to Gram staining, catalase testing, and coagulase testing. Confirmed *S. aureus* isolates were stored at -20°C in the culture bank of Axon Bahar Medical Laboratory for subsequent analysis.

2.4 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar (MHA). Testing was conducted according to the applicable Clinical and Laboratory Standards Institute (CLSI) criteria used in the study. A standardized bacterial suspension equivalent to 0.5 McFarland was prepared and uniformly inoculated onto MHA plates using a sterile cotton swab. The inoculated plates were allowed to dry briefly before antibiotic disks were applied. The antibiotic disks tested included ciprofloxacin, clindamycin, erythromycin, chloramphenicol, cefoxitin, vancomycin, rifampin, gentamicin, doxycycline, methicillin, and trimethoprim. The disks were placed at appropriate distances from each other to prevent overlapping in inhibition zones. The plates were incubated at $35 \pm 2^{\circ}\text{C}$ for approximately 24 hours. Following incubation, inhibition

zones were measured and interpreted according to the applicable susceptibility criteria. Cefoxitin and methicillin resistance were used in the phenotypic assessment of methicillin resistance.

2.5 Preservation of Confirmed Isolates

Confirmed isolates were preserved using glycerol stocks for long-term storage. Overnight bacterial cultures were mixed with glycerol and maintained at -80°C . For recovery, preserved cultures were streaked onto appropriate agar media and incubated. A well-isolated colony was subsequently selected and inoculated into fresh nutrient broth for further analysis.

2.6 Nucleic acid Extraction

Total nucleic acid was extracted from confirmed MRSA isolates using a commercial DNA extraction kit manufactured by Thermo Fisher Scientific, according to the manufacturer's instructions. Briefly, the lysis buffer contained Triton X-100, Tris-HCl (pH 8.0), and EDTA, with lysozyme added immediately before use. Approximately 1.5–2 mL of an overnight MRSA culture was transferred to a microcentrifuge tube and centrifuged at $5,000 \times g$ for 10 minutes. The supernatant was discarded, and the bacterial pellet was resuspended in approximately 180 μL of Gram-positive lysis buffer and incubated at 37°C for 30 minutes. Lysis buffer and Proteinase K were subsequently added, and the suspension was incubated at 56°C for approximately 30 minutes with occasional mixing. RNase A was then added, and the preparation was incubated at room temperature. Ethanol was added before the lysate was transferred to a spin column. The column was centrifuged according to the manufacturer's recommended conditions, followed by washing with Wash Buffer 1 and Wash Buffer 2. The purified DNA was finally eluted using

approximately 200 μL of elution buffer. Extracted DNA was used directly for PCR or stored at -20°C until further analysis.

2.7 DNA Quality Assessment

The extracted DNA was evaluated for quality and quantity using a NanoDrop spectrophotometer and agarose gel electrophoresis. For agarose gel electrophoresis, agarose was prepared in TBE buffer and heated until completely dissolved. Following cooling, ethidium bromide was added for DNA visualization. DNA samples were mixed with loading dye and loaded into the wells of the agarose gel. Electrophoresis was performed at approximately 90 V for 30 minutes. DNA bands were visualized using a UV transilluminator.

2.8 PCR Detection of the *mecA* Gene

The presence of the *mecA* gene was determined using polymerase chain reaction (PCR). A commercially available $2\times$ Green Taq Master Mix (Thermo Fisher Scientific, USA), *mecA*-specific primers synthesized by Macrogen (Korea), PCR-grade water, and a 100-bp DNA ladder were used. PCR reactions were prepared in a final volume of 20 μL . Each reaction contained 10 μL of $2\times$ master mix, 0.5 μL of forward primer, 0.5 μL of reverse primer, 1 μL of DNA template, and 8 μL of PCR-grade water.

2.9 Primers Used for *mecA* Detection

The expected PCR product for the *mecA* gene was 285 bp. The following primers were used to amplify the *mecA* gene as shown in Table 1.

Table 1. Primers Used for *mecA* Detection

Target gene	Primer	Sequence (5'–3')	Expected amplicon	Annealing temperature
<i>mecA</i>	Forward	TGCTATCCACCCTCAAACAGG	285 bp	56.2°C
<i>mecA</i>	Reverse	AACGTTGTAACCAACCCCAAGA		

The PCR reaction mixture was prepared on a clean working surface using sterile PCR tubes to minimize contamination. The reaction components were mixed thoroughly before amplification. The *mecA* amplification was performed using the specified annealing temperature of 56.2°C .

2.9 Agarose Gel Electrophoresis, Visualization and Confirmation of the *mecA* Gene

PCR products were analyzed using 1.5% agarose gel electrophoresis. Agarose was prepared in $1\times$ TBE buffer, heated until completely dissolved, and allowed to cool

before addition of ethidium bromide. The gel was poured into a casting tray containing a comb to form wells. After solidification, the gel was placed in an electrophoresis tank containing TBE buffer. A 100-bp DNA ladder was used as the molecular-size marker. PCR products were loaded into wells and electrophoresed at approximately 120 V for 35 minutes. Following electrophoresis, the gel was examined using a UV transilluminator. DNA bands were compared with the 100-bp molecular-size ladder. Isolates showing a distinct band corresponding to the expected 285-bp *mecA* amplicon were considered positive for the presence of the

mecA gene. Gel images were captured and recorded for subsequent analysis.

2.10 Data Analysis

The microbiological, antimicrobial susceptibility, and molecular results were recorded and analyzed to determine the frequency of MRSA among the *S. aureus* isolates. The distribution of MRSA was assessed according to patient sex and age group. The proportion of *mecA*-positive isolates was calculated among the phenotypically confirmed MRSA isolates. The study included three age categories: 1–20

years, 21–40 years, and 41–75 years. The results were summarized as frequencies and percentages.

3. Results

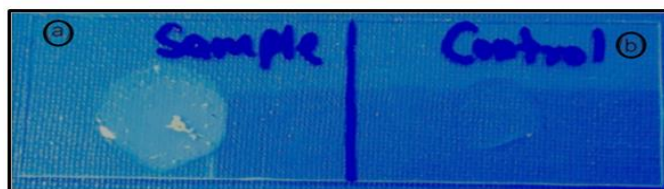
3.1 Isolation and Identification of Bacterial Isolates

Colony morphology of *Staphylococcus aureus* was checked by using Nutrient Agar, Mannitol Salt Agar and blood Agar media as shown in figure 1 (A, B, C, D, E) below. Results of all morphological characteristics and biochemical tests performed are given below in table 2.

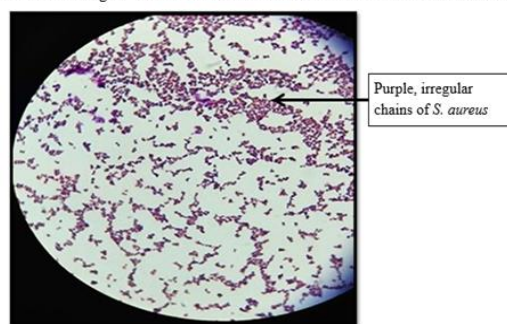
Figure 1: Gram Staining and Biochemical Confirmation of *Staphylococcus aureus* Isolates.



A. *S. aureus* showing yellow colonies on nutrient agar **B.** *S. aureus* showing golden yellow colonies on mannitol salt agar **C.** *S. aureus* on blood agar showing yellow mucoid colonies



D. *S. aureus* coagulase positive isolate showing clumps formation



E. *S. aureus* Purple, irregular chains of spherical under microscope

A. Yellow colonies on nutrients agar. **B.** Golden yellow colonies on mannitol salt agar. **C.** Yellow mucoid colonies on blood agar. **D.** Coagulase positive isolate showing clumps formation. **E.** Purple, irregular chains of spherical *S. aureus* under microscope.

3.2 Antibiotic Susceptibility Patterns

Overall, 100 samples were confirmed as MRSA subjected to antimicrobial susceptibility testing against 11 different antibiotics and results were interpreted accordingly. For total 100 isolates, highest resistance was found for cefoxitin (100%), methicillin (100%) followed by gentamycin

(75%), ciprofloxacin (62%), erythromycin (56%), clindamycin (46%), doxycycline (37%), trimethoprim (36%), chloramphenicol (35%). MRSA isolates were found least resistant to vancomycin (8%) followed by rifampin (15%). A detailed description of antibiotic resistance patterns of MRSA isolates is given in table 3 and figure 4.

Table 2. Morphological characteristics and biochemical tests.

S. No.	Test	Results	
1	Colony morphology	Nutrient Agar	Yellow colonies
		Mannitol Salt Agar (MSA)	Golden yellow colonies
		Blood Agar	Yellow mucoid colonies
2	Gram staining	Gram positive(purple), bunches of grapes (staphylococci)	

3	Coagulase test	Coagulase positive	
4	MRSA detection by disk diffusion assay		Resistant to both cefoxitin and penicillin

Table 3. Antimicrobial susceptibility pattern of MRSA isolates against different antibiotics.

Antimicrobial agent	Resistant (n/N)	Susceptible (n/N)	Total Resistant (%)
Ciprofloxacin	62/100	38/100	62%
Clindamycin	46/100	54/100	46%
Erythromycin	56/100	44/100	56%
Chloramphenicol	35/100	65/100	35%
Cefoxitin	100/100	0/100	100%
Vancomycin	8/100	92/100	8%
Rifampin	15/100	85/100	15%
Gentamycin	75/100	25/100	75%
Doxycycline	37/100	63/100	37%
Methicillin	100/100	0/100	100%
Trimethoprim	36/100	64/100	36%

3.3 MRSA Confirmation Using Disk Diffusion Assay

A total of 150 isolates of *S. aureus* were collected from three tertiary care hospitals of Kabul City and were confirmed for being MRSA. The antibiotics were used according to CLSI guidelines 2026 and cefoxitin antibiotic discs were specifically used for the determination of MRSA isolate to be methicillin resistant. 100 isolates (80%) were confirmed to be MRSA out of 150 suspected isolates and were further considered as MRSA. A representative image of antibiotic testing by Kirby-Bauer disk diffusion method as shown in figure 3 and 4 below.

3.4 Hospital wise distribution of confirmed MRSA isolates

All 150 isolates collected from the three hospitals were subjected to confirmation and 50 isolates were collected from each hospital. Out of 50 isolates collected from Al-Hayat Hospital 40 isolates were found to be MRSA, 35 were MRSA out of 50 isolates collected from Axon Bahar Medical Laboratory. The detailed distribution among the hospitals is shown below in table 4.3 and figure 4.4.

3.5 Gender wise distribution of confirmed MRSA isolates

All 150 isolates were collected along with patient's data and were categorized also on gender basis into male and female groups. Total 75 samples out 150 were collected from male patients and 75 from female patients. 58 (77.33%) isolates were MRSA out of 75 isolates collected from female and 42 (56%) were MRSA out of 75 isolates collected from male patients. The confirmed MRSA distribution based on gender groups is given below in table 5.3.

3.6 Age wise distribution confirmed MRSA isolates

MRSA were distributed differently in different age groups. The highest frequency of MRSA was found in the age group 41-75 years that was 49 (79.03%) out of 62 isolates followed by 1-20 years that was 30 (71.42%) out of 42 isolates. The frequency of MRSA in the age group 21-40 years was 21 (45.65%) out of 46 isolates. Distribution of MRSA in different age groups is explained with the help of table 5.5 and figure 5.11 below.

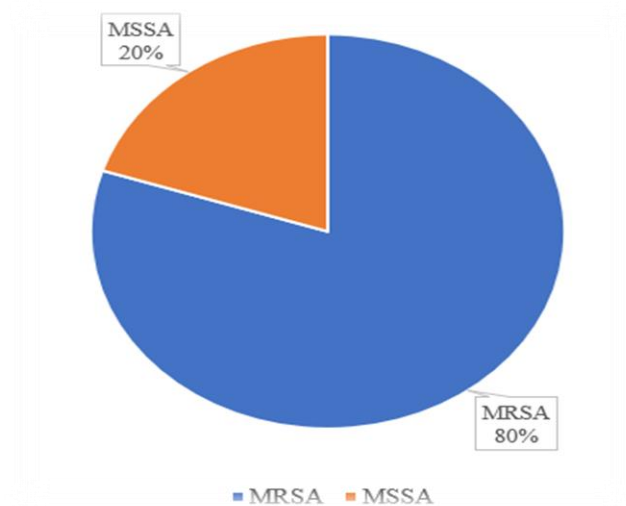


Table 4. Hospital wise percentage of MRSA isolates.

S. No	Hospital	Suspected MRSA	Confirmed MRSA	%
1	Al-Hayat Hospital	50	40	80%

2	Yakta Hospital	50	35	70%
3	Axon Bahar Medical Lab	50	25	50%
	Total	150	100	66.67%

Figure 4. Graphical representation of gender wise MRSA distribution.

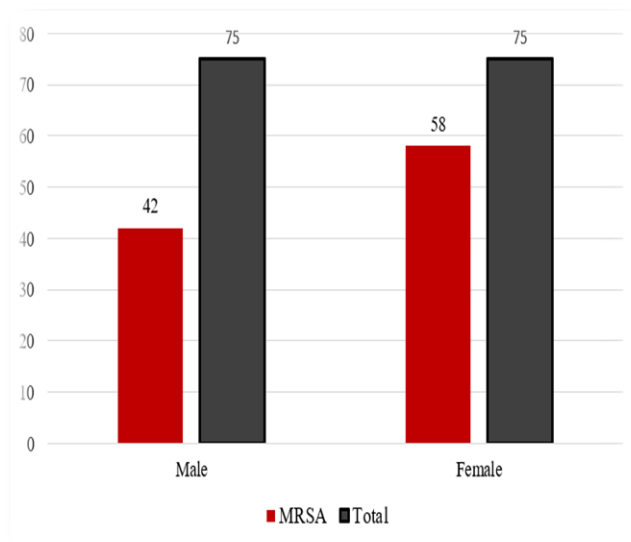
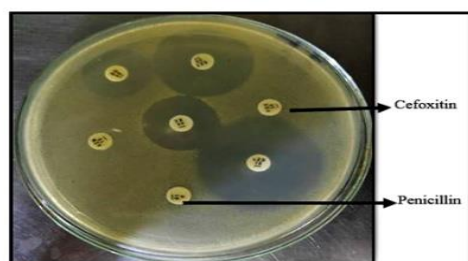


Figure 4: Morphological Confirmation of MRSA, Culture Susceptibility, DNA confirmation through Nanodrop and Gel electrophoresis as well as *mecA* gene confirmation.



A. *Staphylococcus aureus* colonies grown on MHA show resistance both to cefoxitin and penicillin, which confirms them being MRSA.



B. Antibiotic sensitivity patterns of MRSA on Muller Hinton Agar plate.



C. Concentration of DNA obtained through Nano-drop



D. Confirmation of *Staphylococcus aureus* extracted nucleic acid through Gel Electrophoresis



E. Confirmation of *Staphylococcus aureus* *mecA* gene through Gel Electrophoresis

A. MRSA on MHA Agar **B.** Culture Susceptibility of collected and isolated MRSA strains **C.** Concentration of DNA obtained through Nano-drop. **D** Confirmation of extracted DNA by gel electrophoresis. **E.** PCR products on gel under UV light in UV transilluminator showing the presence of 285 bp *mecA* gene bands. The DNA ladder used was a 100 kb plus DNA ladder.

3.8 Detection of *mecA* gene by PCR amplification

For detecting *mecA* gene in the extracted DNA samples, PCR technique was used. The gene was detected in both plasmid and genomic extracted DNA using 1kb plus DNA

Table 5. Age wise distribution of MRSA isolates.

Age group	Collected	MRSA	Percent
(Group 1) 1 year to 20 years	42	30	71.42%
(Group 2) 21 years to 40 years	46	21	45.65%
(Group 3) 41 years to 75 years	62	49	79.03%
Total	150	100	

3.7 Quantification of DNA via nano-drop and DNA Visualization

The DNA extracted from confirmed isolates was analyzed to detect the DNA extraction and to quantify the amount of DNA extracted. For quantification and DNA confirmation nano-drop was used and the concentration of DNA was determined in ng/ul. The results obtained from nano-drop are given in the figure 4 below.

ladder. PCR results showed the presence of *mecA* gene in 86 MRSA isolates out of 100 (86%). The frequency of *mecA* gene in phenotypically confirmed MRSA isolates is explained in table 6. A representative image of gel electrophoresis results showing *mecA* gene as bright bands of 285 bp is given below in figure 4.

Table 6. Detection of *mecA* gene in confirmed MRSA.

S. No.	Total subjected to PCR amplification	MRSA <i>mecA</i> detected in	Percent
	100	86	86%

4. Discussion

Staphylococcus aureus is a very significant pathogen which is responsible for causing various infections both hospital acquired and community associated [14]. *S. aureus* is included in “ESKAPE” (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species*) pathogens for its ability to acquire quick resistance to new antibiotics. MRSA strains of *S. aureus* is resistant to many antibiotics and is the causative agent of different serious infections with high mortality and morbidity in comparison to the infections caused by MSSA [15]. Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a dangerous pathogenic strain of bacteria which is becoming more prevalent in community and hospitals and is strongly multidrug resistant [16].

In the present study, the presence of MRSA isolates was determined by confirming MRSA in suspected isolates of *S. aureus* collected from three different hospitals of Kabul City. A total of 100 isolates out of 150 were found to be MRSA. The MRSA bacteria were determined by double disc synergy method using cefoxitin antibiotic discs. Most of the MRSA were multi-drug resistant and the prevalence of MRSA was 66.67%.

We collected 50 samples from each hospital and confirmed 40 MRSA out of 50 isolates (80%) collected from Al-Hayat Hospital (AHH) Kabul; 35 MRSA were confirmed in 50 isolates (70%) collected from Yakta Hospital Kabul and 25 MRSA were confirmed in 50 isolates (50%) collected from Axon Bahar Medical Laboratory (ABML) Kabul.

Our study has correlation with previous study conducted by Garoy *et al.* [17]. They performed a study on antimicrobial patterns of MRSA isolates in Asmara, Eritrea and included 130 samples and 82 out of them were found to be *S. aureus* (63%). The rate was higher in patients with age less than 18 years and above 60 years. They confirmed 59 MRSA isolates out of 82 *S. aureus* (72%), 7 isolates (8.5%) were confirmed as Methicillin Intermediate *S. aureus* (MISA) and 15 isolates (19%) were confirmed as Methicillin Susceptible *S. aureus* (MSSA). Most of the isolates were from pus samples of burn patients and surgical wounds. Antimicrobial susceptibility testing was performed and 15.9 % isolates (13) were resistant to vancomycin, 11%

isolates (9 were resistant to erythromycin, 1.2% isolates (1) was resistant to gentamycin. The study concluded high prevalence of MRSA and multi-drug resistance which is in agreement with our study.

In present study, we determined the frequency of MRSA in both genders, and we found that the rate of MRSA was higher in females as compared to males. In our study the frequency of MRSA in females was 58 out of 75 (77.33%) as compared to males that was 41 out of 75 (56%). We also determined the frequency of MRSA in different age groups and found that the frequency was higher in the age group of 40 years to 75 years that was 30 out of 42 (79.03%) followed by the age group from 1 year to 20 years that was 49 out of 62 (71.42%) and the least was in the age group from 21 to 40 years that was 21 out of 46 (45.65%). The frequency was higher in elderly people and children where it was slightly lower in young age people ranging from 21 years of age to 40 years.

Similarly, a study performed in Peshawar Pakistan by Ullah *et al.* [18] was also aimed to determine the frequency of MRSA and their antibiotic susceptibility patterns in the region. They included 280 *S. aureus* isolates and the frequency of MRSA was found to be 101 out of 280 (36%). The frequency of MRSA in their study was 36% which was quite insignificant as compared to our study which may possibly be due the samples included as in our study we included only hospital acquired MRSA while they included both hospital acquired and community acquired MRSA isolates. Their sample size may also be a reason for this slightly difference in the frequency with our results. The rate of MRSA was higher in age group of 50 years to 60 years, followed by 20 to 29 years age group. The frequency was higher in females (40%) than males (34%). The rate was higher in community acquired infections (42%) than hospital acquired infections (35%). The MRSA isolates were found 100% resistant to penicillin and cefoxitin antibiotics followed by 99% to erythromycin. The MRSA isolates were found to be 100% susceptible to vancomycin and linezolid antibiotics. 7 isolates were found to be intermediate resistant against vancomycin (VISA). They concluded that MRSA is a potential hazard to public health in the region and should be properly diagnosed and treated to avoid its spread.

Another study performed by Rafiq *et al.* [19] in teaching hospitals of Peshawar was also focused on the frequency of MRSA. They included 390 isolates out of which 180 were confirmed as *Staphylococcus aureus*. Most of *S. aureus* isolates were collected from healthcare facilities individuals (20%) and the remaining were from patients (26.5%). They found 5% of isolates from healthcare individuals as MRSA and 42% as MSSA while in isolates from patients were 4% MRSA and 15% MSSA. Most of the MRSA isolates were from skin and wound infections, followed by pus swabs. Resistance to linezolid was found to be zero, while all MRSA isolates were resistant to oxacillin, amoxicillin, cefotaxime, cefoxitin and cephadrine. They applied simple

control measures for two months and noticed a great reduction in the number of MRSA infections in healthcare personnels and patients as well. They concluded from their results that MRSA greatly vary in their resistance patterns and selection of antibiotics based on culture sensitivity should be done to avoid the spread and controll the threat offered by MRSA to the community and hospitals.

In the present study we also detected the *mecA* gene responsible for the expression of penicillin binding protein 2A (PBP-2A) in MRSA strains. The gene made the bacteria resistant to methicillin along with other potential beta lactam antibiotics and making a global public health threat. We subjected the 100 MRSA isolates to PCR technique by using specific primers and standard conditions. The *mecA* gene was detected in 86 isolates which confirmed them molecularly being MRSA. The detection of *mecA* gene also verify that the MRSA multi-drug resistance is greatly influenced by this gene. We found that 86% MRSA isolated during this study were harboring the *mecA* gene. While those isolates having *mecA* gene were found to be 100% MRSA confirmed which shows the positive relationship of *mecA* gene with MRSA isolates

Siddiqui *et al.* [20] also conducted a study to determine prevalence of *mecA* gene in community acquired MRSA isolates. The study was aimed to investigate the prevalence of *mecA* gene in phenotypically confirmed MRSA and also to determine the antimicrobial susceptibility patterns of *S. aureus* isolates harboring *mecA* gene. They collected 201

isolates of *S. aureus* from different diagnostic laboratories in Karachi and phenotypically confirmed 52% of them as MRSA. But in difference, they performed PCR technique for the amplification of *mecA* gene and found only 36 out of 104 MRSA isolates (35%) [21]. Their study questioned the scientific community that whether the molecular detection of *mecA* is enough for MRSA detection or is there any additional mechanisms which interect with *mecA* gene to develop resistance to β -lactam antibiotics [22]. Their work is in disagreement with our study based on the high frequency of MRSA and also that all those isolates having *mecA* gene were phenotypically confirmed as MRSA.

5. Conclusion

The study concluded that there is a high frequency of MRSA infection in the region with 66.6% *S. aureus* infections being caused by MRSA. It was also concluded from the study that aged people, children and females are more frequently infected with MRSA. Resistance to methicillin antibiotics and other used antibiotics may be developed by *mecA* gene expression but the study also shows that it is possibly associated with other genes and factors which need to be explored and studied. The study can further be extended in different ways like associating *mecA* genes with MSSA infections and determining the association of *mecA* genes with other factors associated with MRSA and multi-drug-resistant *S. aureus*.

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