Introduction

Staphylococcus aureus (S. aureus) is a serious public health concern for both community and hospital-acquired individuals as it causes infections in humans that vary from wound abscesses to life-threatening diseases such as bacteremia, endocarditis, and several others [1]. In the 1960s, methicillin was first utilized as a human medication to treat S. aureus infections but within a year of its clinical usage, MRSA strains emerged [2]. The first case of MRSA was detected in United Kingdom in 1962 and United States (US) in 1968 [3,4]. Methicillin resistance in S. aureus is often associated with the mecA gene that encodes the low-affinity penicillin-binding protein (PBP2a) [5]. MRSA infections are highly prevalent around the world, largely vary among several European countries with a prevalence rate of more than 50% in Malta and Portugal, and less than 5% in Estonia, Denmark, Finland, Norway, Netherlands, and Sweden [6]. In Asian countries, MRSA is quite prevalent, with a hospital-acquired prevalence of 67.4% and a community-acquired rate of 25.5% [7]. Every year in the US 80,000 invasive infections of humans are caused by MRSA, with a mortality rate of 11,000-18,000 people [8,9]. Antibiotic-resistant microorganisms are risking the efficacy of antibiotics as they rapidly increasing around the globe [3]. Due to excessive usage of antibiotics, antibiotic-
resistant bacterial diseases are responsible for more than 35,900 deaths per year and is still a major public health issue in US, with 2.8 million antibiotic-resistant pathogenic infections [10]. Multi-drug resistant (MDR) bacteria are resistant to at least three or more antibiotic classes and are the most prominent trait of MRSA [11]. In Nigeria, β-lactam antibiotics are used for the treatment of MRSA infections, but they are highly (88%) ineffective against them. Even in India and Pakistan, 95% of adults carry β-lactam antibiotic-resistant pathogens [12]. In 2010, a study was conducted at a tertiary care hospital in Mangalore, South India, where a total of 237 isolates were studied, in which 29.1% were methicillin-resistant, while erythromycin, gentamicin, and chloramphenicol resistance were found in 40-50% of isolates. Inducible clindamycin resistance was observed in 18.8% of MRSA strains, with less than 30% resistance to ciprofloxacin and amikacin [13]. Another study from Rawalpindi, Pakistan aimed to determine the prevalence rate of MRSA. Out of 350 staphylococcal isolates, 60.40% were identified as MRSA isolates. All the β-lactam antibiotic drugs were 100% resistant to MRSA followed by nalidixic acid 89.18%, cotrimoxazole 86.48%, erythromycin 85.81%, levofloxacin 80.4%, gentamicin 76.35%, tetracycline 59.45%, ciprofloxacin 44.59%, chloramphenicol 18.24%, and rifampicin 10.13% [14]. Several reports had highlighted a significant proportion of nosocomial and community-acquired MRSA infections in Pakistan [15-18]. The first case of MRSA in Pakistan was discovered in 1989, and the prevalence has been steadily increasing since then. According to researches, the percentage of MRSA isolates grew from 5% in 1989 to 69% in 2020 [17-19]. Therefore, this study aimed to determine the prevalence rate of MRSA, antimicrobial susceptibility profile of S. aureus, MRSA and MSSA isolates to various antibiotics, and its MDR profile. These findings with respect to resistant phenotypes will help in the development of an appropriate hospital antibiotic stewardship policy to reduce the risk of S. aureus-associated infections. It would further highlight the importance of local surveillance in providing useful antibiotic-resistant data that can guide empiric therapy.

M E T H O D S

A total of 106 samples were collected from the microbiology laboratory of HMC, Peshawar, Khyber Pakhtunkhwa (KPK), Pakistan. Various clinical samples including pus, fluids, blood, sputum, throat swab, and tracheal aspirate were collected from December 2020 to May 2021. The clinical isolates were randomly collected from patients who came to the hospital or were already admitted. The randomly collected clinical isolates were processed for bacterial culturing on Mannitol Salt Agar (Oxoid CM0085, England), which is a selective and differential media with 7.5%-10% of salt concentration. Then followed by incubation for 24 hours at 37°C. After the incubation, S. aureus isolates were identified through Gram-staining and isolated colonies were further subjected to biochemical tests including catalase, tube coagulase test and DNase test. S. aureus isolates were further processed for Antibiotic Susceptibility Testing (AST) through the Kirby-Bauer disc diffusion method [20]. Following 11 antibiotics were inoculated on Muller Hinton Agar (MHA) (Oxoid CM0377, England) for antimicrobial testing: penicillin G (P) 10μg, chloramphenicol (C) 30μg, cefoxitin (FOX) 30μg, ciprofloxacin (CIP) 5μg, clindamycin (DA) 2μg, doxycycline (DO) 30μg, erythromycin (E) 15μg, fusidic acid (FD) 10μg, gentamicin (CN) 10μg, linezolid (LZD) 30μg and teicoplanin (TEC) 30μg. Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, 2021 while for teicoplanin CLSI guidelines, 2016 were followed. The breakpoints of fusidic acid were interpreted according to European Committee on Antibiotic Susceptibility Testing (EUCAST) guidelines, 2021. The AST growth suspension was prepared in 5ml normal saline solution with the turbidity adjusted to match the 0.5 McFarland standards to obtain the estimated amount of organism number of 1x10⁶ colony forming units (CFU) per milliliter. After 15 minutes of inoculation, antibiotics discs were placed on MHA, seeded with each isolate, and were cultured at 37°C for 24 hours. After incubation, the antibiotics zones of inhibitions were measured using a ruler and the results were interpreted according to the CLSI and EUCAST guidelines, 2021. For the determination of MRSA, FOX discs of 30μg were used to screen all the S. aureus isolates. S. aureus isolates were grown on MHA agar at 37°C for 18-24 hours with a growth suspension calibrated to 0.5 McFarland standards and inhibition zone equal to or less than 21mm on MHA was considered as MRSA while inhibition zone equal to or greater than 22mm on MHA was considered as MSSA by following CLSI guidelines, 2021. The D-test method was used to determine the inducible clindamycin resistance in MRSA isolates. Briefly, a 0.5 McFarland standards equivalent bacterial culture was seeded on MHA plates, followed by 15mm apart insertion of erythromycin (15μg) and clindamycin (2μg) discs. After that, the plate was incubated for an overnight period and positive inducible clindamycin resistance was determined by a “D” shaped clindamycin zone of inhibition towards an erythromycin disc. For the detection of MDR, Magiorakos et al. [11], definition of non-susceptibility to at least one antimicrobial agent out of three or more antimicrobial classes was used. The chi-square technique was used to establish statistical significance in the age, gender, and specimen type, using GraphPad Prism 9.1.2.226. A p-value
<0.05 was considered statistically significant.

**RESULTS**

**Gram staining**

All of the 106 bacterial isolates were identified as Gram-positive cocci under a light microscope.

**Identification of isolates**

The Gram-positive bacterial isolates on Mannitol Salt Agar plates changed the medium color from pink to yellow, confirming that the bacteria belong Staphylococcus aureus species.

**Biochemical tests**

All the clinical isolates were tested positive for catalase test, tube coagulase test and DNase test.

**Antibiotic susceptibility patterns of S. aureus isolates**

All of the 106 strains were 100% resistant to Penicillin G. High resistance was observed among cefoxitin, ciprofloxacin, and erythromycin i.e., 78.3% (for each) whereas low resistance was found in linezolid 1.9% followed by teicoplanin 2.8% and chloramphenicol 13.2%. Two strains of clindamycin and erythromycin were intermediate while 3 strains (2.8%) were found intermediate to teicoplanin.

For detailed follow Table 1.

**Table 1:** Antibiotic susceptibility profile of S. aureus isolates to various antimicrobial agents

| Classes of Antibiotics | Antibiotics | Drug Susceptibility | MSSA (n%) | MRSA (n%) | p-value |
|------------------------|-------------|---------------------|----------|----------|--------|
| Penicillins            | Penicillin G| Sensitive           | 0        | 0        | 1      |
|                        |             | Intermediate        | 0        | 0        |        |
|                        |             | Resistant           | 98(100)  | 98(100)  |        |
| 2nd generation cephalosporins | Cefoxitin | Sensitive           | 23(21.7) | 83(78.3) |        |
|                        |             | Intermediate        | 23(21.7) | 83(78.3) |        |
|                        |             | Resistant           | 0        | 0        |        |
| Quinolones             | Ciprofloxacin| Sensitive           | 92(86.8) | 14(13.2) |        |
|                        |             | Intermediate        | 14(13.2) | 92(86.8) |        |
|                        |             | Resistant           | 0        | 0        |        |
| Lincosamides           | Clindamycin | Sensitive           | 0(0)     | 59(56.6) |        |
|                        |             | Intermediate        | 59(56.6) | 0(0)     |        |
|                        |             | Resistant           | 0        | 0        |        |
| Tetracycline           | Doxycycline | Sensitive           | 89(84)   | 7(7.6)   |        |
|                        |             | Intermediate        | 7(7.6)   | 89(84)   |        |
|                        |             | Resistant           | 0        | 0        |        |
| Macrolides             | Erythromycin| Sensitive           | 0(0)     | 59(56.6)|        |
|                        |             | Intermediate        | 59(56.6)| 0(0)     |        |
|                        |             | Resistant           | 0        | 0        |        |
| Macrolides             | Fusidic acid| Sensitive           | 80(76.5)| 26(24.5) |        |
|                        |             | Intermediate        | 26(24.5)| 80(76.5)|        |
|                        |             | Resistant           | 0        | 0        |        |
| Aminoglycosides        | Gentamycin  | Sensitive           | 0(0)     | 59(56.6)|        |
|                        |             | Intermediate        | 59(56.6)| 0(0)     |        |
|                        |             | Resistant           | 0        | 0        |        |
| Dizoxolidinones        | Linezolid   | Sensitive           | 0(0)     | 59(56.6)|        |
|                        |             | Intermediate        | 59(56.6)| 0(0)     |        |
|                        |             | Resistant           | 0        | 0        |        |
| Polypeptides           | Teicoplanin | Sensitive           | 0(0)     | 59(56.6)|        |
|                        |             | Intermediate        | 59(56.6)| 0(0)     |        |
|                        |             | Resistant           | 0        | 0        |        |

**Table 2:** Antibiotic susceptibility patterns of MRSA and MSSA isolates to various antimicrobial agents

**Frequencies of MRSA and MSSA**

The frequency of MRSA and MSSA was n=83 (78.3%) and n=23 (21.7%) respectively. MRSA prevalence in males and females were almost the same, n=39 (79.6%) in males and n=44 (77.2%) in females. On the other hand, the prevalence of MSSA in males and females were n=10 (20.4%) and 13 (22.8%) respectively. According to the numbers of specimens, MRSA was most frequent in pus n=56 (80%), followed by fluids n=22 (75.9%), blood n=4 (100%) and tracheal aspirate n=1(100%). On the other hand, MSSA was most frequently found in pus n=14 (20%) followed by fluids n=7(24.1%), sputum n=1(100%) and throat swab n=1(100%). Age-wise distribution showed that MRSA vs MSSA between ages 51-60 years was 81% vs 19%, 41-50 was 80% vs 20%, 11-20 and 21-30 was 70% vs 30% for both respectively. For detailed descriptions of the frequencies of MRSA and MSSA strains, follow Table 3.

**Table 3:** Frequencies of MRSA and MSSA (Total number of S. aureus isolates), X2 (Chi-square), p-value <0.05 is considered
statically significant

**Resistant phenotype of S. aureus**

Ninety-four (88.67%) of the isolates were MDR. MDR strains ranged from resistance to three classes of antibiotics (n=10, 9.43%) to 9 classes of antibiotics (n=25, 23.58%). The high resistance rate for MDR was observed among 4–5 classes of antibiotics (n=25, 23.58%). The detailed resistance of the MDR pattern is given in Table 4.

| Antibiotics | No. of resistant strains | Percentage of resistant strains |
|-------------|--------------------------|--------------------------------|
| P           | 3                        | 2.83                           |
| P, CIF      | 4                        | 8.50                           |
| P, FOX      | 5                        |                                |
| P, DA, E    | 2                        | 8.50                           |
| P, CIF, DA  | 1                        |                                |
| P, FOX, FD  | 2                        |                                |
| P, FOX, CIP | 4                        |                                |
| P, CIF, E, FD | 1              | 21.70                          |
| P, E, CIP, FD | 1           |                                |
| P, DA, E, CN | 1          |                                |
| P, CIF, DA, E | 7          |                                |
| P, FOX, DD, E | 1          |                                |
| P, FOX, DA, E | 4          |                                |
| P, FOX, CIP, E | 6          |                                |
| P, FOX, CIP, CN | 2        |                                |
| P, FOX, CIP, DA, E | 2 | 18.88                          |
| P, FOX, DA, DD, E | 2          |                                |
| P, FOX, CIP, DA, E, CN | 10  |                                |
| P, CIP, DA, E, FD, CN | 10  |                                |
| P, FOX, C, CIP, DA, E, FD | 1 |                                |
| P, FOX, CIP, DA, E, CN | 1 |                                |
| P, FOX, C, CIP, DA, E, CN | 1 |                                |
| P, CIP, DA, DD, E, FD, TEC | 1 |                                |
| P, FOX, CIP, DA, DD, E, FD | 1 |                                |
| P, FOX, CIP, DA, E, FD, CN | 3 |                                |
| P, FOX, CIP, DA, DD, E, CN | 1 |                                |
| P, FOX, C, CIP, DA, E, FD, CN | 1 | 2.83                           |
| P, FOX, CIP, DA, DD, E, FD, CN | 1 |                                |
| P, FOX, C, CIP, DA, E, FD, CN, TEC | 1 | 1.88                           |
| P, FOX, C, CIP, DA, DD, E, FD, TEC | 1 | 0.94                           |

**Table 4:** Percentages of resistance pattern of S. aureus isolates to various antibiotics

P (Penicillin G), FOX (Cefoxitin), E (Erythromycin), FD (Fusidic acid), CIP (Ciprofloxacin), C (Chloramphenicol), DO (Doxycycline), DA (Clindamycin), CN (Gentamycin), TEC (Teicoplanin), LZD (Linezolid)

**D I S C U S S I O N**

S. aureus is one of the most leading causes of hospital and community-acquired infections around the world due to its enhanced virulence and continuous development of antibiotics resistance [17,21]. The major findings of current study were MDR-MRSA, MRSA resistance in the age group of 2 and 9 months, and teicoplanin intermediate strains. Multi-drug resistance in MRSA has been a major issue around the world, resulting in ineffective therapy and higher treatment costs [6]. The current study highlighted a high (88.67%) number of MDR S. aureus isolates which was in line with the recent reports of 68% from Karachi, Pakistan [18], 83.8% from Kabul, Afghanistan and an earlier report of 71.7% from Zaria, Nigeria [22,23]. Among 106 S. aureus isolates, MRSA was observed in 78.3% of samples which was a little high compared to the reports of 66.7% from Rahim Yar Khan-Punjab, Pakistan and 65% from Islamabad, Pakistan [24,17]. Gender-wise distribution showed that MRSA were highly prevalent in both males and females i.e., 79.6% and 77.2% respectively which was in correspondence with another study from Rawalpindi, Pakistan [25]. There were no statistically significant differences observed in MRSA prevalence by age, gender, and specimen type. A high (100%) prevalence of MRSA were found in the age group of 61–69 years. One strain (1.2%) of MRSA was isolated from the blood of the age group 2 months and one (1.2%) from the 9 months. According to the number of isolates, the current study reported that MRSA was most frequent in pus (80%) which was high compared to a study of 36.7% from Peshawar, Pakistan [26]. The antibiotic resistance rate demonstrated by S. aureus isolates to Penicillin was 100% which was in line with the previous findings from Pakistan [14,26], Afghanistan [22] and India [13]. Resistance exhibited to cefoxitin by S. aureus isolates in this study was 78.3% which was quite greater than the previous reports of 47.54% from Islamabad, Pakistan [16] and 66.7% from Rahim Yar Khan-Punjab, Pakistan [24]. Several reports of 100% resistance to cefoxitin antibiotics were also been previously observed in various cities (Rawalpindi, Karachi and Peshawar) of Pakistan [14,18,24]. The current study documented 25.30% resistance to MRSA against fusidic acid which was lower than several other studies of 40.6% [24], 53.1% [27] and 66.7% [28] from Pakistan. In the present study, MRSA resistance exhibited by ciprofloxacin was 79.52% which was quite greater compared to 44.59% [14] and 48% [24]. This study indicates a lower rate of MRSA resistance to doxycycline i.e., 19.28% which was very low compared to the previous studies of 41.6% [24] and 46.8% [27] from Pakistan, and 81.4% from Afghanistan [22]. In the current study, MRSA resistance to clindamycin was 59.04% which correlated with the findings of 60.1% [15] and 51% [27] from Pakistan. Two (2.41%) isolates of MRSA were found intermediate to clindamycin. Interestingly 84.3% of MRSA strains were resistance to erythromycin in the present study which were quite higher compared to the previous
reports of 28.90% [25] and 46% from Pakistan [24], and 23% from Afghanistan [22] but lower compared 99.01% [26] from Pakistan. This study reported 12.04% of inducible clindamycin resistance in MRSA isolates which was almost in line with one of the study from Peshawar Pakistan that reported 15.84% of inducible clindamycin resistance strains in MRSA isolates [26]. The present study documented that MRSA was highly sensitive to linezolid and teicoplanin which can be used as a drug choice to treat MDR-MRSA infections. Resistance exhibited by MRSA to teicoplanin was 2.41% which was quite lower compared to 25% [18]. Zero percent resistance to teicoplanin has also been observed in various researches from Pakistan [15,27] and Turkey [29] while 2.41% resistance to linezolid was exhibited to MRSA isolates which was very low compared to 21.1% [25] and 24.1% [16]. Several reports of 0% resistance to linezolid were also been observed in Pakistan [15,26], India [30,31] and Turkey [29].

**Conclusions**

The current study found a high prevalence rate of MRSA in the patients of HMC Peshawar, KPK, Pakistan. The MDR-MRSA is a major public health concern in Peshawar. This bacterium can disseminate in the community and as well as in health facilities and can cause severe infectious diseases. Linezolid and teicoplanin were highly susceptible to MRSA and could be the drugs of choice for treating MRSA infections. To further understand the epidemiology and molecular causes of antibiotic resistance in MRSA, more research is needed in different regions of Pakistan.

**Conflicts of Interest**

The authors declare no conflict of interest.

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