Proceedings of the Pakistan Academy of Sciences: B Life and Environmental Sciences 62(1): 79-87 (2025)

ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(62-1)1082

Pakistan Academy of Sciences

Research Article

Prevalence and Antimicrobial Susceptibility Patterns of Methicillin-Resistant *Staphylococcus Aureus* (MRSA) in Patients from District Bahawalpur

Muhammad Faizan Munawer[1], Khawar Ali Shahzad[1,2*], Munazzah Marryum[1], Mavra Saleem[1], and Shabana Ramzan[1]

[1]Department of Zoology, The Islamia University of Bahawalpur, Bahawalpur, 63100, Pakistan
[2]Shanghai Fourth People's Hospital, and School of Medicine, Tongji University, Shanghai, 200120, China

Abstract: Methicillin-resistant *S. aureus (MRSA)* is an opportunistic bacterium causing a lot of infections, including infections of skin and soft tissues, endocarditis, pneumonia and bacteremia leading to death globally. This study aimed to determine the Methicillin-resistant *S. aureus* (MRSA) incidence and the antimicrobial pattern of MRSA among patients at a tertiary care hospital of Bahawalpur. Clinical samples (blood, sputum, catheter tips, pus, blood, urine, body fluid, wound, nasal and throat swabs) were collected from the hospital and transported to the microbiology laboratory. Culture and sensitivity testing was done in the laboratory to find out the antimicrobial susceptibility of *S. aureus* according to CLSI guidelines 2021. Among 622 collected specimens, 82 *S. aureus* isolates were found, and MRSA was found in 55 samples. MRSA was more prevalent in males as compared to females. In urine, a high incidence of MRSA was found. In age groups, a high prevalence was seen in the 61–70-year age group, and the lowest prevalence was seen in the 0–10-year age group. In OPD, the *S. aureus* prevalence was 39%. Among wards, the highest prevalence of MRSA was recorded in the ICU. Among *S. aureus* isolates, high resistance was shown by penicillin, cefoxitin and Sulphamethoxazole. Linezolid was found to be highly susceptible to *S. aureus* isolates. Linezolid, vancomycin, Fusidic acid, teicoplanin and clindamycin were the most effective antibiotics for treating infection caused by MRSA. The current study also noticed high resistance of bacteria to numerous antibiotics, revealing the significance of monitoring antibiotic consumption.

Keywords: *S. aureus,* MRSA, MSSA, Kirby Bauer, Antimicrobial Susceptibility.

1. INTRODUCTION

Staphylococcus aureus (S. aureus) is a well-known bacterium in humans, causing nosocomial infections in both community and hospital including skin and soft tissue infections, osteomyelitis, endocarditis, impetigo, and many other fatal diseases. It is a gram-positive, non-motile, spherical grape-like cluster and coccoid bacterium with a diameter of about 1 µm [1]. It is the common cause of death worldwide, indicating a poor diagnosis and treatment, having 20% to 40% fatality rate [2]. *S. aureus* is found asymptomatically on human body parts, i.e., skin and its glands, and mucous membranes, including healthy people's noses and guts as it can cause a variety of symptomatic infections, as it does not always live in harmony with humans [3]. In 1942, the first strain of *S. aureus* that was resistant to penicillin was discovered, and the penicillinase (β-lactamase) enzyme made antibiotics ineffective [4]. After methicillin was developed in late 1950, used to treat *S. aureus* infection, first methicillin resistant *S. aureus* was diagnosed in Europe in 1960 as it became resistant to β-lactam drug such as methicillin and amoxicillin [5]. The main cause of methicillin resistance is penicillin binding proteins (PBP2a) with low affinity, which is expressed by the mecA gene, on the staphylococcal

Received: July 2024; Revised: February 2025; Accepted: March 2025
* Corresponding Author: Khawar Ali Shahzad <khawar7bar@yahoo.com>

chromosomal cassette mec (SCCmec) that results in resistance to antimicrobial β-lactams [6]. MRSA is classified into two types, community associated (CA-MRSA), and hospital associated MRSA (HA-MRSA) categories based on where the infection originated [7]. Individuals who have recently been hospitalized with serious diseases, the aged and those who reside in long-term healthcare facilities have more chances of HA-MRSA infections [8]. Health Care Workers (HCWs) are significant contributors to MRSA transmission in hospitals, as there are higher chances of colonization by continuous exposure to the hospital environment [9]. Virulent elements, including the toxic shock syndrome toxin 1(TSST-1), gamma-toxin, Panton Valentine leukocidin (PVL) and secretion of many exotoxins and enterotoxins by MRSA, cause infection such as cutaneous abscesses and mainly soft and skin tissue infection (SSTI), which if left untreated leads to critical diseases that may be fatal when CA-MRSA enters the cardiac, respiratory and skeletal systems. Due to increased virulence and transmissibility, CA-MRSA can spread terribly, whereas HA-MRSA cannot [10].

In Pakistan, the 1st MRSA case was spotted in 1989 [11], and its prevalence gradually increased [12]. In 2024, a 5.5% prevalence of MRSA was recorded in Faisalabad [13], and 89% was recorded in Lahore [14]. In Europe, the prevalence of infection caused by MRSA was found to be less than 5% in Finland, Netherlands, and Denmark while in Portugal and Malta, the incidence rate was more than 50% [15]. In Asian countries, the incidence of hospital and community linked MRSA was 67% and 25% respectively [16]. In the United States, there was a high rate of prevalence of about 60% in intensive care units with MRSA. About 80,000 infections were caused by MRSA with fatality rate of 14-20% annually [17]. Almost all strains of MRSA are highly resistant to various antimicrobial classes, including lincosamide, β-lactam drugs, aminoglycosides, and macrolides. Because of this, multidrug-resistant MRSA (MDR-MRSA) reduces the effectiveness of available treatments for *Staphylococcus* infections and causes severe outcomes, worsening the situation globally and becoming a severe health challenge for doctors [18]. Patients with staphylococcal infection are challenging to treat with the emergence of MRSA strains, lengthening hospital stays, increasing costs, and making them resistant to available antimicrobial drugs MRSA transmission and spread can be reduced and controlled by identifying health care workers (HCWs) who are colonized and are MRSA transmission vectors, using hand hygiene, and taking other preventive measures [19]. Unfortunately the decline in developing novel antibiotics in the face of quickly rising resistance to existing antibiotics exacerbates the situation. Due to resistance to glycopeptides, vancomycin has recently emerged as a source of worry, posing a problem in treating of tenacious MRSA infections [20]. Only a few antibiotics including Linezolid, Teicoplanin and Tigecyclines were effective against MRSA [21]. In recent years, the enormous incidence of MRSA and limited treatment options have emerged as quiet threats to public health. Therefore, this study aimed to investigate the methicillin-resistant *S. aureus*, its prevalence, demographic factors, and the antibiotic susceptibility of MRSA.

2. MATERIALS AND METHODS

This study was carried out at the pathology and endocrinology section of Quaid-E-Azam Medical College (QMC) and spanned from September 2022 to February 2023 and involved the collection of different clinical samples from the patients of the outdoor department (OPD) and indoor departments of Bahwal Victoria Hospital (BVH) using aseptic techniques. This study was conducted after approval from the Ethical Review Committee of our institution (letter No. IUB/ERC/25/2022), in 2022 and the simple random sampling technique was adopted to collect specimens according to the biosafety standards and international safety rules after informed consent to patients. Six hundred and twenty-two (622) different clinical samples, including blood, sputum, catheter tips, pus, blood, urine, body fluid, wound, nasal and throat swabs, were collected in highly sterilized, leak-proof, and dried containers under the supervision of medical officers using aseptic techniques before antibiotic use. From OPD, 73 samples were collected, and 61 samples were collected from the patients admitted in urology, nephrology, medical, surgical, ENT, paediatrics, paediatrics surgical, ICU, and CCU, respectively. Regardless of age and gender, patients belong to different regions of Bahawalpur and Bahwalnagar districts and was admitted or came to hospital were included in this study. From this study, patients who refused to participate and patients who experienced nasal bleeding were excluded because

rolling the swab could worsen the bleeding. To avoid duplication and respecting patient's privacy, a specific code was given to each sample.

Blood samples were collected in BACT/ ALERT blood vials and inoculate in an automated BACT/ALERT 3D for 5-7 days at 37 °C for microbial identification. Sterile swab sticks swab the wound and nose back into its case. All randomly collected samples were inoculated on different media, i.e., Blood, MacConkey and CLED (OXOID, UK), according to the sample type for culturing and incubated for 24 hours at 37 °C. After 24 hours, the bacterial growth on media was seen, and the identification of *S. aureus* was done by following standard identification procedures, including gram staining, the morphology of the colony, and biochemical test, i.e., catalase test, coagulase test and DNase test [22]. After confirmation, *S. aureus* was processed by using the Kirby-Bauer disc diffusion method for antimicrobial susceptibility testing [23]. Muller Hinton agar plates were used in antimicrobial susceptibility testing. Different types of antibiotics were used to test antibiotic sensitivity of *S. aureus,* including Penicillin (P) 10 µg, Cefoxitin (FOX) 30 µg, Erythromycin (E) 30 µg, Clarithromycin (CLR) 15 µg, Moxifloxacin (MXF) 5 µg, Teicoplanin (TEC) 30 µg, Sulphamethoxazole (SXT) 25 µg, Vancomycin (VA) 30 µg, Fusidic acid (FD) 10 µg, Linezolid (LNZ) 30 µg, Clindamycin (DA) 10 µg, and Tobramycin (TOB) 10 µg. On Muller Hinton agar, pure *S. aureus* isolated colonies were streaked, and antibiotic discs were impregnated, which were then incubated for 24 hours at 37 °C. After 24 h of incubation, the inhibition zone around the discs was measured using a scale and explained according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) [24] and Clinical and Laboratory Standards Institute (CLSI) recommendations [25]. Using Cefoxitin (FOX) 30 µg, *S. aureus* resistant to methicillin were identified with inhibition zone of 21 mm or less were MRSA while greater than 21 mm were considered MSSA. CLSI criteria from 2016 were used for teicoplanin, while results were demosntrated following the Clinical and Laboratory Standards Institute (CLSI) criteria from 2021. Categorical data were summarized using frequency and percentage using Microsoft Excel 365. The association between participant characteristics and *S. aureus* was analyzed using chi-square tests. A two-tailed method was employed with the significance threshold set at $\alpha = 0.05$. SPSS V.27 were used for statistical analysis.

3. RESULTS

3.1. Gram Staining

Under the light microscope, 82 (13%) isolates were found in Gram-positive cocci out of six hundred and twenty-two (622) specimens.

3.2. Biochemical Test

These gram-positive bacterial isolates were then confirmed for *S. aureus* by various biochemical test, i.e., catalase, coagulase and DNase test which showed positive results.

3.3. *S. aureus* Antibiotic Susceptibility

Among all (82) isolates, Linezolid was highly sensitive to 95%, while Teicoplanin, Vancomycin, Fusidic acid and Clindamycin were sensitive to 90% of all isolates. Penicillin was found to be highly resistant (76%). Cefoxitin was resistant to 67% of isolates, 62% resistant to Sulphamethoxazole, 59% to Clarithromycin, and 55% of all isolates were resistant to Erythromycin, Tobramycin, and moxifloxacin shown 34% and 21% resistance to isolates respectively (Table 1).

3.4. Prevalence of MRSA and MSSA

The prevalence of MRSA and MSSA was n = 55 (67%) and n = 27 (33%) respectively. Among the eighty-two (82) *S. aureus* isolates, 25 (31%) blood samples, 32 (39%) pus, 6 (7%) body fluids, and 19 (23%) urine samples were included. Out of 25 blood samples, 14 (56%) were MRSA, 11 (44%) were MSSA. Among 32 pus samples, 22 (69%) MRSA, 10 (31%) MSSA were included, six samples of body fluids included 4 (67%) MRSA, and 2 isolates of (33%) MSSA while 19 urine samples had 15 (79%) MRSA samples and 4 (21%) samples of MSSA were found. Among 82 *S. aureus* samples, 32 (39%) samples were from OPD and 50 (61%) samples were from different wards including urology, surgical ward, peads surgical ward, peads ward, medical ward, surgical ward, nephrology, ENT, ICU and CCU. From OPD, 21 (66%) isolates of MRSA and 11 (34%) isolates of MSSA were found. In urology, 87% (7) MRSA isolates were

Table 1. Antibiotic pattern of *S. aureus* isolates.

Classes of Antibiotics	Antibiotics	Resistant (%)	Sensitive (%)	P-value
Penicillin's	Penicillin (P)	62 (76)	20 (24)	0.305
Cephalosporins	Cefoxitin (FOX)	55 (67)	27 (33)	0.210
Macrolides	Erythromycin (E)	45 (55)	37 (45)	0.056
	Clarithromycin (CLR)	48 (59)	34 (41)	0.104
	Fusidic acid	9 (11)	73 (89)	0.438
Quinolones	Moxifloxacin (MFX)	17 (21)	65 (79)	0.353
Polypeptides	Teicoplanin (TEC)	8 (10)	74 (90)	0.448
	Vancomycin (VA)	8 (10)	74 (90)	0.448
Sulphonamides	Sulphamethoxazole (SXT)	51 (62)	31 (38)	0.150
Oxazolidinones	Linezolid (LNZ)	4 (5)	78 (95)	0.483
Lincosamide	Clindamycin (DA)	9 (11)	73 (89)	0.438
Aminoglycosides	Tobramycin (TOB)	28 (34)	54 (66)	0.210

found, 60% in surgical wards, 33% in peads surgical wards, and 42% in peads wards. MRSA was found more prevalent (100%) in nephrology, medical wards and in ICU respectively, followed by 75% in CCU and 0 isolate was found in ENT.

Out of 82 *S. aureus* isolates, 54 (66%) were male samples, and 28 (34%) were from female. The prevalence of MRSA among males was (n = 39) 72% and in females (n = 16), 57% of MRSA isolates were included. Conversely, MSSA was (n = 15) 28% prevalent in males and 43% (n = 12) in females. In different age groups, the frequencies of MRSA and MSSA was 35% vs 64% in 0-10 years, 57% vs 42% in 11-20 years, 63% vs 36% in 21-30, 55.5% vs 44% in 31-40 years age group. The highest prevalence of MRSA was seen in the 61-70 years age group, following 51-60- and 41-50-year age groups with frequencies of 88% and 80%, respectively . The data shows no significance for gender, specimen types, and collection site. Although, age may influence the *S. aureus* index with the p-value 0.013.

4. DISCUSSION

S. aureus is the primary cause of infections acquired in hospitals and communities worldwide due to its increased virulence and the ongoing emergence of antibiotic resistance. The last twenty years have seen an upsurge in the global spread of MRSA [26, 27]. In current research, the prevalence of *S. aureus* was 13% among collected samples, of which the MRSA and MSSA found 67% vs 33% prevalent. It is quite common in many nations, with prevalence rates greater than 80% in Latin America and rising to 19% in Australia [28]. In Pakistan, the prevalence of MRSA is increasing due to improper usage of antibiotics, as reported in the previous study, which was 78.3% in Hayatabad and 100% in Peshawar [29], 66% in Rahim Yar Khan [30, 31], and 52% prevalence of MRSA was reported in Karachi [32] and 56% was reported in a recent study [33]. The prevalence of MRSA was reported at 81% in hospitals in Egypt and 54.85% in Uttar Pradesh [34, 35]. In the current study, the prevalence of MRSA among *S. aureus* isolates was 67% which is close to the previous reported study. Among the total collected samples, the MRSA prevalence in the current study was 9%, which is agreed with the results of [21] in which a 10.4% prevalence of MRSA was reported and 6.37% in Peshawar [36]. The prevalence of MSSA in previous studies was 22% in Peshawar [31], and 35% in Islamabad [37], almost agreeing with the results of the current study in which the prevalence of MSSA was 33%.

The MRSA and MSSA prevalence in gender were studied, and in males, the prevalence of MRSA was higher than in females. The higher frequency of MRSA in males than in females was also reported by other researchers in Pakistan and India [21, 38-40]. A high (100%) frequency of MRSA was seen in the 61–70-year age group, followed by 88% in 51–60, 80% in 41–50, and the lowest prevalence was seen in the 0–10-year-old group, which was 35% in

Table 2. Detailed prevalence of *S. aureus* (MRSA & MSSA) among different age groups, specimen types, Gender-wise and in other Wards and OPD.

Variables	*S. aureus*	MRSA	MSSA	$\bar{X}$±SD	χ^2 (P-value)
AGE					16.213 (0.013)
0-10	17	6 (35.29)	11 (64.71)	2.69±2.897	-
11--20	7	4 (57.14)	3 (42.86)	15.71±3.302	-
21-30	11	7 (63.64)	4 (36.36)	26.45±2.733	-
31-40	9	5 (55.56)	4 (44.44)	34.88±2.315	-
41-50	15	12 (80)	3 (20)	47±1.732	-
51-60	17	15 (88.24)	2 (11.76)	55.58±2.526	-
61-70	6	6 (100)	0 (0)	68.33±3.777	-
GENDER					1.898 (0.168)
Male	54	39 (72)	15 (28)	27±16.97	-
Female	28	16 (57)	12 (43)	14±2.828	-
SPECIMENS					2.642 (0.450)
Blood	25	14 (56)	11 (44)	12.5±2.121	-
Pus	32	22 (69)	10 (31)	16±8.485	-
Fluid	6	4 (67)	2 (33)	3±1.414	-
Urine	19	15 (79)	4 (21)	9.5±7.778	-
WARDS & OPD					14.864 (0.095)
Urology	8	7 (88)	1 (13)	4±4.242	-
Surgical ward	10	6 (60)	4 (40)	5±1.414	-
Peads surgical ward	3	1 (33)	2 (67)	1.5±0.707	-
Peads ward	12	5 (42)	7 (58)	6±1.414	-
Nephrology	6	6 (100)	0	3±4.242	-
Medical ward	4	4 (100)	0	2±2.828	-
ICU	2	2 (100)	0	1±1.414	-
CCU	4	3 (75)	1 (25)	2±1.414	-
ENT	1	0	1 (100)	0.5±0.707	-
OPD	32	21 (66)	11 (34)	16±7.07	-

this research. A high frequency (100%) of MRSA was seen in the 61–69-year-old group in a previous study [31], 61%-68% was studied in different age groups [41]. The maximum prevalence of MRSA among isolates was seen in urine (79%) followed by pus (69%), fluid (67%), and blood (56%) in the current study. In Peshawar, the prevalence of MRSA in pus, fluid and blood was 35%, 42% and 48%, respectively [42], in blood, 100% MRSA prevalence was found [31] and 72% in pus samples [36]. In current study, the MSSA prevalence in blood was 44%, 31% in pus, 33% in fluid, and 21% in urine. The results reported from Narowal exhibit 53% prevalence in pus and 28% in blood [38].

S. aureus is responsible for 70% of ICU infections, many of which are MRSA [43]. The current study's prevalence was 100% in the ICU because of immune deficiency and serious diseased patient, who had more chances of infection. The

prevalence of MRSA in OPD was reported 63% in a study conducted in Rahim Yar Khan that closely agreed with this study's result in which the prevalence rate was 66%. In surgical ward, 39% prevalence of MRSA was reported. In different studies, the *S. aureus* resistance to penicillin was high (100%) but the findings of this study disagreed with the resistance exhibited by *S. aureus* to penicillin, which was 76%. Cefoxitin was found resistant in 67% of the isolates in this study which was in line with the results of study conducted in Rahim Yar Khan and Peshawar [30, 31]. Although, cefoxitin was 100% in some previous studies reported in different cities of Pakistan [32]. In Islamabad, cefoxitin resistance was low in compared to the present study [44]. Erythromycin was found resistant in 55% of *S. aureus* isolates. In previous studies, 99% and 84% resistance were documented in Peshawar [31, 42], 80.3% in Faisalabad [13], 78% in Rawalpindi [21], and 65% in Karachi, showing high resistance as compared to the present study [32]. Although low resistance of Erythromycin was also reported in some previous studies compared to the current study, 29% in Rawalpindi [41], and 46% in Rahim Yar Khan [30] of Pakistan. Clarithromycin was resistant to 59% of isolated strains in this study and was not correlated with the study conducted in Narowal and Peshawar [45, 46]. Fusidic acid resistance in the present study was lower as compared to the previous studies in Pakistan, revealing high resistance, 25% in Peshawar [31], 41% in Rahim Yar Khan [30], and 42% in Narowal [38]. In the current study the resistance of Clindamycin to MRSA isolates was 11%, which was not correlated with previous studies because of the resistance being too high. Clindamycin showed 51%-60% resistance to MRSA in previous studies from Pakistan [31]. In this study, Linezolid was only resistant to 5% of *S. aureus* strain and 95% susceptible to MRSA. In a former study conducted in Rawalpindi the Linezolid was 89.5% susceptible [21], and same trend was seen in [47]. Moxifloxacin was susceptible to 80% of isolates in a study conducted in Lahore [48]. The sensitivity of moxifloxacin to *S. aureus* isolates in the current study was noted 79% which was quite close to the findings of previous studies conducted in Lahore. The present study demonstrated that teicoplanin was susceptible to 90%. In Pakistan and Turkey, 100% susceptibility was observed in some previous studies [49, 50]. Vancomycin was susceptible to 90% of *S. aureus* isolates and resistant to 10%. The resistance in this study was a little higher than the previous study reported in Pakistan and different countries of the world. Vancomycin showed zero percent resistance and had outstanding results against MRSA in previous studies of Pakistan and India [36, 51]. In Islamabad, 97% sensitivity of Vancomycin was observed against MRSA [21], 95% in Iran [52] and 100% in Kabul [53] and 95% was reported in Ethiopia [54]. In our study, high resistance was shown by penicillin and cefoxitin subsequent to Sulphamethoxazole and macrolides.

The study explores important concerns in the healthcare field through the assessment of antibiotic resistance. However, it lacks the comprehensive classification of samples, and the lack of consideration of comorbidities as an aspect of risk factors. This study also does not utilize modern molecular methods of microbial analysis, such as whole-genome sequencing. Therefore, future studies should provide insight into the molecular mechanisms of resistance to fight against infections.

5. CONCLUSIONS

The rising trend of MRSA infections highlights the necessity of effective and strict infection control strategies. In the current study, over half (65%) of the isolates were MRSA, which was more prevalent in older adults and in males. Penicillin, cefoxitin, and sulfamethoxazole were highly resistant. Linezolid, clindamycin, fusidic acid, vancomycin, and teicoplanin were most susceptible in our research and can be used to treat infection caused by MRSA. Culture and sensitivity tests should be done to treat any infection before prescribing antibiotics and avoiding self-medication in order to control emerging resistance of microbes to commercially available antibiotics. Furthermore, research in different Pakistan regions is needed to gain insight into the epidemiology and molecular mechanisms of antibiotic resistance in MRSA.

6. ETHICAL STATEMENT AND PARTICIPANTS CONSENT

This study was approved by the Ethical Review Committee of "The Islamia University of Bahawalpur, Pakistan" under the letter No. IUB/ERC/25/2022. Written informed consent was obtained from all participants before their inclusion in the study.

7. ACKNOWLEDGEMENTS

The authors are thankful to the officials and paticipants for providing the facilities for sample and data collection and processing. This research is completed without financial support from any public, commercial, or non-profit funding agencies.

8. CONFLICT OF INTEREST

The authors declare no conflict of interest.

9. REFERENCES

1. S.Y. Tong, J.S. Davis, E. Eichenberger, T.L. Holland, and V.G. Fowler Jr. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews* 28(3): 603-661 (2015).
2. A. Hassoun, P.K. Linden, and B. Friedman. Incidence, prevalence, and management of MRSA bacteremia across patient populations - a review of recent developments in MRSA management and treatment. *Critical Care* 21(1): 211 (2017).
3. K. Loewen, Y. Schreiber, M. Kirlew, N. Bocking, and L. Kelly. Community-associated methicillin-resistant Staphylococcus aureus infection: Literature review and clinical update. *Canadian Family Physician* 63(7): 512-520 (2017).
4. C.H. Rammelkamp and T. Maxon. Resistance of Staphylococcus aureus to the Action of Penicillin. *Proceedings of the Society for Experimental Biology and Medicine* 51(3): 386-389 (1942).
5. E.J. Benner and F. Kayser. Growing clinical significance of methicillin-resistant Staphylococcus aureus. *The Lancet* 2(7571): 741-744(1968).
6. R. Hassan, R. Barwa, M.M. EL-Sokkary, and D. Ashraf. Virulence characteristics of methicillin resistant Staphylococcus aureus isolated from different clinical sources. *New Egyptian Journal of Microbiology* 48: 14-31 (2017).
7. A.M. Algammal, H.F. Hetta, A. Elkelish, D.H.H. Alkhalifah, W.N. Hozzein, G.E.S. Batiha, N. El-Nahhas, and M.A. Mabrok. Methicillin-Resistant Staphylococcus aureus (MRSA): one health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. *Infection and Drug Resistance* 13: 3255-3265 (2020).
8. N.K. Sharma, R. Garg, S. Baliga, and G. Bhat. Nosocomial infections and drug susceptibility patterns in methicillin sensitive and methicillin resistant Staphylococcus aureus. *Journal of Clinical and Diagnostic Research* 7(10): 2178-2180 (2013).
9. R. Khanal, P. Sah, P. Lamichhane, A. Lamsal, S. Upadhaya, and V.K. Pahwa. Nasal carriage of methicillin resistant Staphylococcus aureus among health care workers at a tertiary care hospital in Western Nepal. *Antimicrobial Resistance and Infection Control* 4: 39 (2015).
10. A.S. Lee, H. de-Lencastre, J. Garau, J. Kluytmans, S. Malhotra-Kumar, A. Peschel, and S. Harbarth. Methicillin-resistant Staphylococcus aureus. *Nature Reviews Disease Primers* 4: 18033 (2018).
11. B. Ashiq and A.K. Tareen. Methicillin resistant Staphylococcus aureus in a teaching hospital of Karachi-a laboratory study. *Journal of the Pakistan Medical Association* 39(1): 6-9 (1989).
12. M. Riffat. Prevalence, antibiotic susceptibility pattern and demographic factors related to methicillin resistant Staphylococcus aureus in Lahore, Pakistan. *International Journal of Microbiology and Advanced Immunology* 2(3): 45-48 (2014).
13. A. Taqveem, M.H. Rasool, B. Aslam, F. Mujahid, A. Ibrar, H. Ejaz, A.E. Abdalla, Y. Alruwaili, M. Alruwaili, K. Abosalif, Z. Nawaz, and M. Khurshid. Methicillin-resistant Staphylococcus aureus among students: nasal carriage rate, contributing factors, and antimicrobial susceptibility. *Medicina (Kaunas)* 60(10): 1590 (2024).
14. A. Khatoon, S.F. Hussain, S.M. Shahid, S.K. Sidhwani, S.A. Khan, O.A. Shaikh, and A.J. Nashwan. Emerging novel sequence types of Staphylococcus aureus in Pakistan. *Journal of Infection and Public Health* 17(1): 51-59 (2024).
15. R. Köck, K. Becker, B. Cookson, J. van Gemert-Pijnen, S. Harbarth, J. Kluytmans, M. Mielke, G. Peters, R. Skov, and M. Struelens. Methicillin-resistant Staphylococcus aureus (MRSA): burden of disease and control challenges in Europe. *Eurosurveillance* 15(41): 19688 (2010).
16. J.H. Song, P.R. Hsueh, D.R. Chung, K.S. Ko, C.I. Kang, K.R. Peck, J.S. Yeom, S.W. Kim, H.H. Chang, and Y.S. Kim. Spread of methicillin-resistant Staphylococcus aureus between the community and the hospitals in Asian countries: an ANSORP study. *Journal of Antimicrobial Chemotherapy* 66(5): 1061-1069 (2011).
17. F. Nikkhahi, S. Robatjazi, M. Niazadeh, A. Javadi, G. Shahbazi, P. Aris, S.A. Marashi, and N. Emam. First detection of mobilized colistin resistance mcr-1 gene in Escherichia coli isolated from livestock and sewage in Iran. *New Microbes and New Infections* 41: 100862 (2021).

18. S. Lakhundi and K. Zhang. Methicillin-Resistant Staphylococcus aureus: Molecular characterization, evolution, and epidemiology. *Clinical Microbiology Reviews* 31(4): 10-128 (2018).
19. L. Li, M.R. Yeaman, A.S. Bayer, and Y.Q. Xiong. Phenotypic and genotypic characteristics of methicillin-resistant Staphylococcus aureus (mrsa) related to persistent endovascular infection. *Antibiotics (Basel)* 8(2): 71 (2019).
20. M. Alseqely, M. Newton-Foot, A. Khalil, M. El-Nakeeb, A. Whitelaw, and A. Abouelfetouh. Association between fluoroquinolone resistance and MRSA genotype in Alexandria, Egypt. *Scientific Reports* 11(1): 4235 (2021).
21. M.N. Noorulamin, Z.B. Zarafsah, A. Janjua, A. Iqbal, S. Humerah, and P.A. Shaiq. Future trends in the treatment of MRSA in Pakistan. *Journal of Islamabad Medical & Dental College* 11(2): 96-102 (2022).
22. J.D. Turnidge. Susceptibility Test Methods: General Considerations. In: Manual of Clinical Microbiology. M.L. Landry, J.H. Jorgensen, K.C. Carroll, M.A. Pfaller, D.W. Warnock, S.S. Richter, and G. Funke (Eds.). *Wiley, ASM Press* pp. 1246-1252 (2015).
23. A. Bauer. Antibiotic susceptibility testing by a standardized single diffusion method. *American Journal of Clinical Pathology* 45(4): 493-496 (1966).
24. E. Matuschek, D.F. Brown, and G. Kahlmeter. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. *Clinical Microbiology and Infection* 20(4): 255-266 (2014).
25. R. Humphries, A.M. Bobenchik, J.A. Hindler, and A.N. Schuetz. Overview of Changes to the Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing, M100, 31st Edition. *Journal of Clinical Microbiology* 59(12): e0021321 (2021).
26. E. Avershina, V. Shapovalova, and G. Shipulin. Fighting antibiotic resistance in hospital-acquired infections: current state and emerging technologies in disease prevention, diagnostics and therapy. *Frontiers in Microbiology* 12: 707330 (2021).
27. A.A. Khan, J. Farooq, M. Abid, and R. Zahra. Assessment of inducible clindamycin resistance and Hyper Variable Region (HVR) of mecA gene in clinical staphylococci. *Pakistan Journal of Medical Sciences* 36(2): 136 (2020).
28. S. Baig, A. Rhod Larsen, P. Martins Simões, F. Laurent, T.B. Johannesen, B. Lilje, A. Tristan, F. Schaumburg, B. Egyir, and I. Cirkovic. Evolution and population dynamics of clonal complex 152 community-associated methicillin-resistant Staphylococcus aureus. *mSphere* 5(4): e00226-20 (2020).
29. W. Abbas, F. Parveen, H. Zaman, and J. Shah. Analysis of hospital-acquired mrsa infections in pus samples: a comprehensive discussion. *Insights-Journal of Health and Rehabilitation* 3(3): 214-221 (2025).
30. M.S. Hussain, A. Naqvi, and M. Sharaz. Methicillin resistant Staphylococcus aureus (MRSA); prevalence and susceptibility pattern of methicillin resistant staphylococcus aureus (mrsa) isolated from pus in tertiary care of district hospital of rahim yar khan. *The Professional Medical Journal* 26(01): 122-127 (2019).
31. L.U. Rehman, A.A. Khan, P. Afridi, S.U. Rehman, M. Wajahat, and F. Khan. Prevalence and antibiotic susceptibility of clinical staphylococcus aureus isolates in various specimens collected from a tertiary care hospital, Hayatabad, Peshawar, Pakistan.: Antibiotic susceptibility of clinical staphylococcus aureus isolates in various specimens. *Pakistan Journal of Health Sciences* 3(4): 105-110 (2022).
32. T. Siddiqui, I.N. Muhammad, M.N. Khan, S. Naz, L. Bashir, N. Sarosh, R. Masood, A. Ali, S. Fatima, and T. Naqvi. MRSA prevalence and susceptibility pattern in health care setups of Karachi. *Pakistan Journal of Pharmaceutical Sciences* 30(6): 2417-2421 (2017).
33. S. Batool, W. Saleem, K. Ahmad, and S.-u.H. Khan. Antibiotic resistance and antibiotic resistance genes in Staphylococcus aureus isolates from hospital patients in Islamabad, Pakistan. *Pakistan Journal of Zoology* 57(2): 1-10 (2025).
34. A.A.E. Alfeky, M.M. Tawfick, M.S. Ashour, and A.N.A. El-Moghazy. High prevalence of multi-drug resistant methicillin-resistant Staphylococcus aureus in tertiary Egyptian hospitals. *The Journal of Infection in Developing Countries* 16(05): 795-806 (2022).
35. P.P. Preeja, S.H. Kumar, and V. Shetty. Prevalence and characterization of methicillin-resistant Staphylococcus aureus from community- and hospital-associated infections: a tertiary care center study. *Antibiotics (Basel)* 10(2): 197 (2021).
36. W. Ali, S. Zaman, Z. Subhan, A. Razaq, M. Nabi, M. Khattak, N. Naeem, S.A. Bangash, and I. Ullah. Methicillin-Resistant Staphylococcus aureus (MRSA) epidemiology and antibiotics susceptibility profile isolated from different clinical

samples in tertiary care hospital: mrsa epidemiology and antibiotics susceptibility profile. *Pakistan BioMedical Journal* 5(5): 108-112 (2022).

37. A.A. Khan, A. Ali, N. Tharmalingam, E. Mylonakis, and R. Zahra. First report of mecC gene in clinical methicillin resistant S. aureus (MRSA) from tertiary care hospital Islamabad, Pakistan. *Journal of Infection and Public Health* 13(10): 1501-1507 (2020).
38. S.N. ul-Hassan, K. Farva, and G.A. Bhutta. Multidrug resistant Staphylococcus aureus; its incidence and antibiotic sensitivity pattern. *The Professional Medical Journal* 28(11): 1590-1594 (2021).
39. M. Rashmi, S. Krishna, and S. Qayoom. Prevalence of MRSA among Clinical Isolates of Staphylococcusaureus and its Antibiotic Susceptibility Pattern at a Tertiary Care Hospital. *International Journal of Current Microbiology and Applied Sciences* 6: 747-749 (2017).
40. T.M. Tariq, K. Anwar, M.D. Iqbal, S.F. Dogar, and N. Saeed. Frequency of methicillin-resistant staphylococcus and antibiogram profile of staphylococcus aureus recovered from different clinical samples. *International Journal of Pathology* 22(1): 24-29 (2024).
41. S. Khan, F. Rasheed, and R. Zahra. Genetic polymorphism of agr locus and antibiotic resistance of Staphylococcus aureus at two hospitals in Pakistan. *Pakistan Journal of Medical Sciences* 30(1): 172-176 (2014).
42. A. Ullah, M. Qasim, H. Rahman, J. Khan, M. Haroon, N. Muhammad, A. Khan, and N. Muhammad. High frequency of methicillin-resistant Staphylococcus aureus in Peshawar region of Pakistan. *Springerplus* 5(1): 600 (2016).
43. E. Avershina, V. Shapovalova, and G. Shipulin. Fighting antibiotic resistance in hospital-acquired infections: current state and emerging technologies in disease prevention, diagnostics and therapy. *Frontiers in Microbiology* 12: 707330 (2021).
44. A.A. Khalil-ur-Rahman, A. Aziz, M. Daud, and I. Khan. Antibiotic susceptibility patterns of methicillin resistant Staphylococcus aureus at national institute of health sciences, Islamabad, Pakistan. *World Journal of Zoology* 10(4): 318-322 (2015).
45. M.S. Al-Zoubi, I.A. Al-Tayyar, E. Hussein, A. Al-Jabali, and S. Khudairat. Antimicrobial susceptibility pattern of Staphylococcus aureus isolated from clinical specimens in Northern area of Jordan. *Iranian Journal of Microbiology* 7(5): 265 (2015).
46. A.T. Khan, K. Jamil, N. Farooqi, M. Bilal, and I. Hussain. Frequency of methicillin resistant Staphylococcus aureus in patients referred from other specialities and its antimicrobial susceptibility and resistance pattern. *Journal of Medical Sciences* 22(1): 28-31 (2014).
47. A. Fatima, I. Sajid, S. Riaz, and M. Saeed. Detection of methicillin resistant Staphylococcus aureus using mec a, ribotyping and antibiogram profile of Pakistani clinical isolates. *The Professional Medical Journal* 26(06): 993-999 (2019).
48. W. Ghias, M. Sharif, F.A. Yazdani, and M. Rabbani. Isolation and identification of Methicillin and Vancomycin resistance Staphylococcus aureus from pus samples of injured skin patients in Lahore, Pakistan. *Biomedical Letters* 2(2): 103-112 (2016).
49. F. Bari, R. Wazir, M. Haroon, S. Ali, I. Ali, H. Rahman, A. Ullah, A.M. Khattak, and M. Qasim. Frequency and antibiotic susceptibility profile of MRSA at lady reading hospital, Peshawar. *Gomal Journal of Medical Sciences* 13(1): 62-65 (2015).
50. F.A. Shah, S.U. Din, and W.A.W.M. Khan. Frequency and antimicrobial susceptibility pattern of Methicillin resistant Staphylococcus aureus in open fractures. *Journal of Surgery Pakistan (International)* 21(2): 62-66 (2016).
51. V. Pai, V.I. Rao, and S.P. Rao. Prevalence and antimicrobial susceptibility pattern of Methicillin-resistant Staphylococcus aureus MRSA. Isolates at a tertiary care hospital in Mangalore, South India. *Journal of Laboratory Physicians* 2(2): 82-84 (2010).
52. H. Motamedi, B. Asghari, H. Tahmasebi, and M.R. Arabestani. Identification of hemolysine genes and their association with antimicrobial resistance pattern among clinical isolates of Staphylococcus aureus in West of Iran. *Advanced Biomedical Research* 7(1): 153 (2018).
53. H.M. Naimi, H. Rasekh, A.Z. Noori, and M.A. Bahaduri. Determination of antimicrobial susceptibility patterns in Staphylococcus aureus strains recovered from patients at two main health facilities in Kabul, Afghanistan. *BMC Infectious Diseases* 17(1): 737 (2017).
54. T. Dilnessa and A. Bitew. Prevalence and antimicrobial susceptibility pattern of methicillin resistant Staphylococcus aureus isolated from clinical samples at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia. *BMC Infectious Diseases* 16: 398 (2016).