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Original article

Molecular identification of resistant genes in methicillin resistant Staphylococcus aureus among cardiac patients

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Abstract. *Background and aim: Staphylococcus aureus*, a prevalent cause of nosocomial and cardiac infections, particularly methicillin-resistant strains (MRSA), poses significant public health concerns due to limited treatment options. The current study of *mec*A and *van*A genes confers resistance in *S. aureus* in Open Heart surgery patients. *Methods:* Among hundred clinical samples (pus) were processed tested for antimicrobial susceptibility, detecting MRSA using cefoxitin disc diffusion. Vancomycin Minimum Inhibitory Concentration (MIC) was determined, and chromosomal DNA was isolated for *mec*A and *van*A gene analysis. *Results:* In this study, bacterial growth was found in 87% of the total specimens tested where *S. aureus* (68.9%) was the most common. All 60 *S. aureus* isolates were Penicillin-resistant, and the next most resistant antibiotics were erythromycin (96.6%), gentamicin (92.6%), while the highest sensitivity was shown by vancomycin (100%). MRSA was found in 70% of the sixty *S. aureus*. Out of 42 MRSA isolates, 4.7% were vancomycin intermediate *Staphylococcus aureus* (VISA) and the rest 95.2% were vancomycin sensitive *Staphylococcus aureus*. MRSA were not resistant to the drug vancomycin. The amplified *mecA gene* (167bp) was found in 61.6% (37/60) of the 60 *S. aureus*. None of the *S. aureus* strains possessed the *vanA* gene. *mec*A gene was found in 88.0% of 42 MRSA isolates and 100% of VISA isolates and five-methicillin-sensitive *S. aureus* (MSSA) were deficient in the *mec*A gene. *Conclusions:* High MRSA prevalence in cardiac patients increases antibiotic resistance, requiring expansion of diagnostic facilities and regular monitoring. (www.actabiomedica.it)

Key words: MRSA, VISA, MSSA, *Staphylococcus aureus*, cardiac patients

Background

The *Staphylococcus aureus*, a bacterium responsible for various illnesses, including life-threatening sepsis, is characterized by its virulence factors, which are released by bacteria and other disease-causing microorganisms. (1). MRSA, an opportunistic bacterium, causes various illnesses (2). Nosocomial infections being the most common cause of infection and death in heart surgery patients (3). Cardiovascular infections pose a significant risk due to their potential damage to the heart's valves, endocardium, myocardium, and pericardium (4). Cardiothoracic surgery often leads to surgical site infection, primarily caused by *S. aureus*, *viridans streptococci*, coagulase-negative staphylococci, and Gram-negative *bacilli*, with *S. aureus* being the predominant Gram-positive bacterium (5).

The *S. aureus* is a common cocci found in both community and hospital settings (6). Major heart surgery is frequently linked to S. aureus surgical site

infections and the patient's endogenous microbiota (5). MRSA, a methicillin-resistant strain of S. aureus, was first identified in the UK in the 1960s due to the use of methicillin in healthcare (7). MRSA is responsible for over 50% of *S. aureus* infections currently (2). MRSA has developed antibiotic resistance worldwide, initially to penicillin and later to methicillin, flucloxacillin, oxacillin, cephalosporins, monobactams, carbapenems, cephems, and combinations of β-lactams and β-lactamase inhibitors (8). The *mec*A gene, located in the genomic site of the Staphylococcus cassette chromosome, encodes PBP2a (penicillin-binding protein), a movable extrinsic genetic element that blocks the active site from binding β-lactams, resulting in lower affinity for β-lactams than PBP2, generated by MSSA (2), This gene affects *S. aureus* pathogenicity (9). Vancomycin, a primary treatment for severe MRSA infections, has contributed to the VRSA phenotype's emergence due to its inappropriate use and the degradation of precursors by D-Ala-D-lac or D-Ala-D-Ser substitutes, for which vancomycin has a low affinity (10). The mecA gene, commonly used as an MRSA marker, indicates β-lactam (11). The expression of *van*A gene indicates potential VRSA resistance to glycopeptides (12).

MRSA identification relies on phenotypic techniques, with PCR-based genotypic approaches being the "gold standard" due to their ability to detect the *mec*A gene. Rapid detection methods include Oxacillin disc diffusion (ODD) culture and antibiotic screening tests, while agar screening techniques include mannitol salt agar (MSA), minimum inhibitory concentration assays (MIC), and agar dilution tests (13). PCR has reduced the time and effort needed to detect MRSA, potentially aiding in infection prevention and management (14), as evidenced by similar bacterial growth patterns observed in Pakistani clinical settings (15). It is believed that the widespread use of antibiotics for the past six decades has created resistant strains of bacteria due to miss use of antibiotics. This inefficient maintenance of health facilities has resulted in an increase in the death ratio associated with cardiac infections. The current study identified and characterized MRSA from clinical samples of cardiac patients. Resistance genes from the isolates were also found out by using molecular techniques. This finding will minimize the misuse of antibiotics and will be helpful for the physicians to prescribed drug of choice for cardiac patients.

Materials and methods

Study design

This was a cross sectional study conducted from August to December, 2022 among patients attending Peshawar Institute of Cardiology and Cardiology Department of Rehman Medical Institute, Peshawar, Pakistan. Totally, 100 clinical specimens of pus were obtained and examined using established microbiological techniques (16).

Inclusion/Exclusion criteria

Hospitalized patients (both genders) of open-heart surgery of all age (up to 90 years old) were included while other cardiac patients or who have recent used antibiotics were excluded in the study.

Samples collection and processing

During the study 100 clinical samples (pus) through sterile swab were collected from hospitalized patients and were labeled with date, time and patient history and were handled according to the standard microbiological procedure (16). In addition to signs and symptoms, information regarding previous infection, any underlying disease and antibiotics used previously were also collected from patients.

Culturing and identification of the isolates

Blood agar, MacConkey agar and Chocolate agar media were used for culturing. The isolated strains were identified through culture characteristics and colony morphology on culture media through Gram staining and biochemical tests (17).

Antimicrobial susceptibility test of isolated organisms

To check antimicrobial susceptibility of *S. aureus* Kirby Bauer disc diffusion technique was followed

(18), and for the results interpretation CLSI, 2022 criteria were followed (19). The antibiotics discs used were Amikacin (30µg), Co-trimoxazole (25µg), Cefoxitin (30µg), Clindamycin (2µg), Rifampicin (5µg), Chloramphenicol (30µg), Erythromycin (15µg), Levofloxacin (5µg), Gentamicin (10µg), Linezolid (30µg), Penicillin (30µg), Teicoplanin (30µg), Vancomycin(15µg), Minocycline (30µg), Moxifloxacin (50µg) and Doxy (30µg).

Detection of methicillin resistance

Using the Kirby-Bauer disc diffusion technique, cefoxitin (FOX) (30µg) was used to screen *S. aureus* isolates for methicillin resistance (18). MRSA forms a zone of inhibition around cefoxitin discs of less than 21mm, whereas MSSA forms greater than 22mm according to the CLSI (2022) criteria (19).

Identification of vancomycin intermediate

To identify vancomycin-intermediate and resistant bacteria, the minimum inhibitory concentration (MIC) of *S. aureus* isolates was measured. The MIC of vancomycin for MRSA was calculated using CLSI (2022) recommendations (19). Vancomycin-incorporated plates with various doses ranging from 0.0625 to 32g/ml were prepared. Each isolate included a positive control, and each test also contained *S. aureus* (ATCC 25923) with a known MIC as a measure of antibiotic potency. Vancomycin's MIC value of less than 2µg/ml in *S. aureus* was regarded susceptible; between 4 and 8µg/ml was considered intermediate resistance; and more than 16µg/ml was classified resistant (20).

Multidrug resistant S. aureus determination

Antibiotic-resistant bacteria are categorized as MDR if they are resistant to three or more types of antibiotics (21).

Culture preservation

The culture that proved *S. aureus* was preserved in 500mL of tryptic soy broth (TSB) by inoculating a single colony and was placed in Eppendorf, identified by its sample number, and allowed to develop there till next day. The culture was vortexed for 30 seconds to thoroughly mix in 500mL of 70% glycerol in TSB and cultures were kept at -80°C.

DNA extraction

An overnight culture was used for DNA extraction which was confirmed by morphological and biochemical tests. The previously reported methodology for DNA extraction was followed (22).

PCR optimization of resistant genes

PCR protocols were optimized to amplify the target gene. For this purpose, a known pair of primers for *mec*A gene were (Forward): (5′-ACT GCT ATC CAC CCT CAA AC-3′) and (Reverse): (5-′CTG GTG AAG TTG TAA TCT GG-3′) of amplicon size 163bp (23). Similarly, primers for *van*A gene were (Forward): (5′-ATG AAT AGA ATA AAA GTT GC-3′) and (Reverse): (5′-TCA CCC CTT TAA CGC TAA TA-3′) of amplicon size 1032bp (24) were used. Gradient PCR reactions was carried out to detect different antibiotic resistance genes. PCR amplification reactions were performed in a 25µL volume using a master mix comprising 200µM of dNTPs (dATP, dCTP, dGTP and dTTP) and 120nM of each primer. 0.5 U/µL Taq polymerase was added to 1 PCR buffer, 25mM $MgCl_2$, and 1µL of DNA. For the *mec*A gene, amplification reactions were carried out under the following thermal and cycling conditions: Three minutes of denaturation at 94°C, followed by 35 cycles of 45 seconds denaturation at 94°C, 30 seconds annealing at 55°C, 3 minutes of extension at 72°C and two minutes of final extension at 72°C. The *van*A gene was initially denatured at 95°C for two minutes, followed by 35 cycles of denaturation at 95°C for one minute, annealing at 56°C for one minute, extension at 72°C for one minute and final extension at 72°C for five minutes.

Gel electrophoresis

Following amplification, 10µL of the PCR product was combined with 2-3µL of loading dye and injected into wells. A DNA marker (Thermo scientific

100bp ladder) was added in one well. The 1% gel was then run for 55 minutes at 90 volts in 1X Tris-borate-EDTA (TBE) buffer in the gel tank (Bio-Rad). After the specified time, the PCR products were examined with a UV trans-illuminator (25).

Control strains used in the study

Quality control was conducted using *S. aureus* ATCC 29213 (*mec*A negative), *S. aureus* ATCC 49476 (*mec*A positive), and *Enterococcus faecalis* ATCC 51299, a vancomycin-resistant strain. To confirm the PCR method was carried out correctly, sterile water (negative), DNA that was known to be positive, and negative controls from earlier extractions (positive) were utilized as PCR controls.

Statistical analysis

The data was interpreted using a Microsoft Excel spreadsheet and after that, it was analyzed through the Statistical Package for the Social Sciences (SPSS) v.26 and Chi-square test was used for association for antibiotic susceptibility patterns.

Results

Isolated bacteria's growth pattern

Out of 100 samples bacterial growth was detected in 87% (87/100) of the tested samples. Male patients (63.2%; 55/87) outnumbered female patients (36.7%; 32/87) in our research. A total of (42.5%; 37/87) of cardiac patients were from age 41 to 60 years, followed by 21 to 40 years of age (33.3%; 29/87), >60 years age were (18.3%; 16/87) and 0 to 20 were (5.7; 5/87). Among the 87 hospitalized cardiac patients of inpatient departments (51.7%; 45/87), bacterial infection rates were higher than those among outpatient departments (48.2%; 42/87) (p =0.01) (Table 1).

Distribution of bacterial genera among isolates with a positive culture

A total of 4 distinct bacterial genera were found among the 87 culture-positive isolates. The most common bacteria among them in cardiac patients were *S. aureus* (68.9%; 60/87), *K. pneumoniae* (12.6%; 11/87), *E. coli* (10.3%; 9/87) and *Enterococcus* spp. (8.0%; 7/87) (Figure 1).

Table 1. Demographic characters and positive bacterial isolates

Characters	Processed Samples	Culture Positive	
		Number	%age
Clinical Sample Processed	100	87	87%
Type of Clinical Sample			
Pus	100	87	87%
Gender			
Male	63	55	63.2%
Female	37	32	36.7%
Age group in years			
<20	8	5	5.7%
21-40	35	29	33.3%
41-60	38	37	42.5%
>60	19	16	18.3%
Types of Patients			
Inpatients department (IPD)	53	45	51.7%
Outpatients department (OPD)	47	42	48.2%

Antimicrobial testing of S. aureus

Totally 60 positive *S. aureus* isolates were subjected for antimicrobial sensitivity and resistant testing. Antimicrobial resistant observed against 16 antibiotics, happened to be penicillin resistant and the next most resistant antibiotics were erythromycin (96.6%; 58/60), gentamicin (92.6%; 55/60), while vancomycin sensitive (100%; 60/60) were observed as shown in Table 2.

Antimicrobial testing of methicillin-resistant S. aureus

Out of a total, 60 *S. aureus* isolates, based on cefoxitin resistance, 70% (n=42) were MRSA. They were further processed for antibiotics sensitivity, it occurred that all of the MRSA isolates were resistant to penicillin (100%; 42/42), followed by erythromycin (97.6%; 41/42), gentamicin (95.2%; 40/42), while

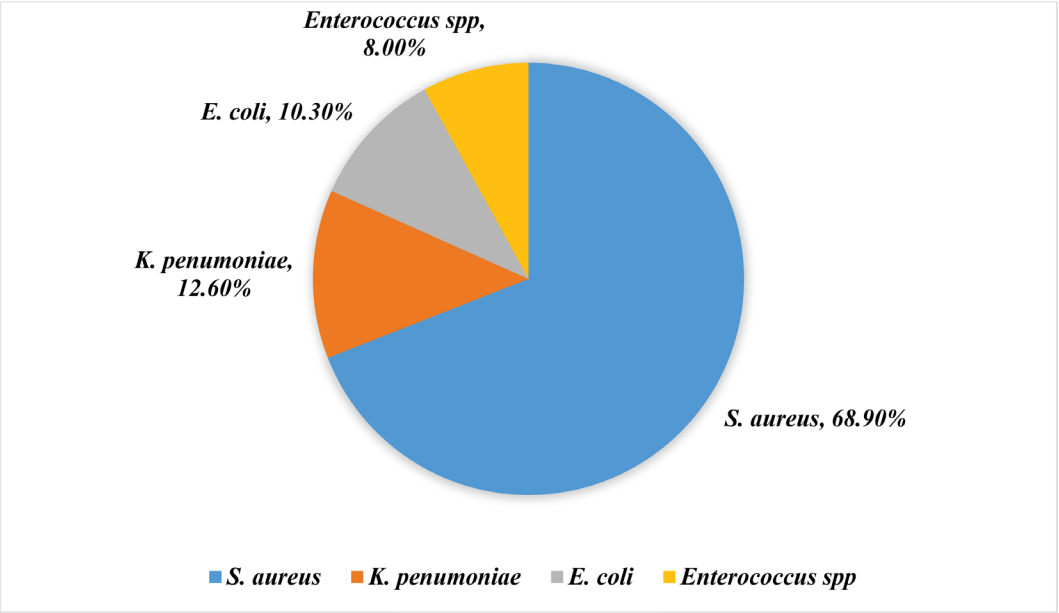

Figure 1. Distribution of different bacterial isolates.

Table 2. Antimicrobial pattern of S. aureus and MRSA

Antibiotics	*S. aureus* (n=60)		MRSA (n=42)	
	Sensitive Frequency(%age)	Resistant Frequency(%age)	Sensitive Frequency(%age)	Resistant Frequency(%age)
Penicillin	0 (0)	60 (100)	0 (0)	42 (100)
Erythromycin	2 (3.3)	58 (96.6)	1 (2.3)	41 (97.6)
Gentamicin	5 (8.3)	55 (92.6)	2 (4.7)	40 (95.2)
Clindamycin	31 (51.6)	29 (48.3)	11 (26.1)	29 (69.0)
Cefoxitin	14 (6.6)	42 (70)	0 (0)	42 (100)
Ciprofloxacin	22 (36.6)	38 (63.3)	9 (21.4)	33 (78.5)
Vancomycin	60 (100)	0 (0)	42 (100)	0 (0)
Amikacin	17 (28.3)	43 (71.6)	7 (16.6)	35 (83.3)
Chloramphenicol	35 (58.3)	25 (41.6)	25 (59.5)	17 (40.4)
Moxifloxacin	17 (28.3)	33 (55)	11 (26.1)	31 (73.8)
Rifampicin	31 (51.6)	29 (48.3)	19 (45.2)	23 (54.7)
Doxycycline	19 (31.6)	41 (68.3)	11 (26.1)	31 (73.8)
Minocycline	9 (15)	51 (85)	7 (16.6)	35 (83.3)
Co-trimoxazole	26 (43.3)	34 (56.6)	17 (40.4)	25 (59.5)
Linezolid	43 (71.6)	17 (28.3)	35 (83.3)	7 (16.6)
Levofloxacin	7 (11.6)	53 (88.3)	5 (11.9)	37 (88.0)

MRSA, MSSA, VSSA and VISA prevalence based on clinical isolates, gender, age and patient type.

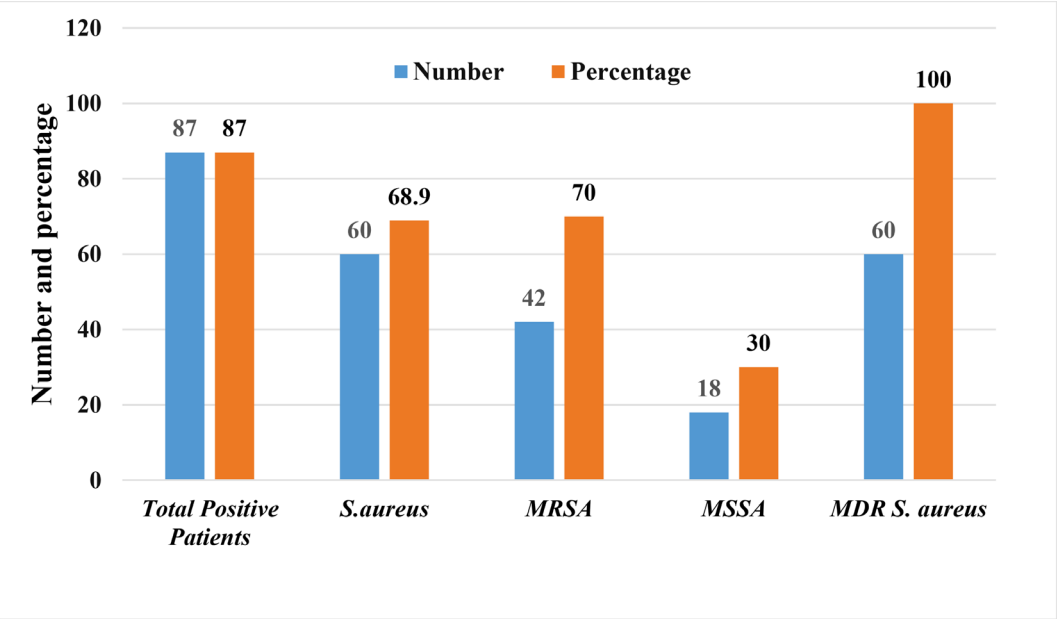

Figure 2. Prevalence of *S. aureus*, MSSA, VSSA and MDR *S. aureus.*

vancomycin sensitive (100%; 42/42) were observed and are shown in Table 2.

Among the 60 *S. aureus* isolates, 70% (42/60 isolates) were MRSA and 30% (18/60) were MSSA. 60 *S. aureus* isolates were made up of 100% (60/60) from pus (Figure 2). Infection with *S. aureus* was more common in male (71.6%; 43/60) than female (28.3%; 17/60). Pus was the most common source of MRSA isolates among the 42 total isolates (Table 3), MRSA isolates were more common in-patient department samples taken from males (61.1%; 31/42 patients) than from females (26.1%; 11/42). Patients between 41 to 60 years old had the highest percentage of MRSA isolates (40.4%; 17/42) followed by those between the ages of 21 to 40 were (30.9%; 13/42), >60 years were (21.4% (9/42), and 0 to 20 years were (7.1%; 3/42). Similarly, MRSA was present in 76.1% (32/42) of the patients from the in-patient's department. 4.76% (2/42) of the 42 MRSA isolates were VISA, whereas the remaining (95.2%; 40/42) were VSSA. Two isolates of VISA were taken from in-patient's department (Table 3).

Identification of vancomycin intermediate (MIC)

There were 42 MRSA isolates, of which 4.7% (2/42) were VISA, while the rest (95.2%; 40/42) were vancomycin resistant. The drug vancomycin did not show resistance to MRSA.

Prevalence of MDR S. aureus *isolates*

The most common bacteria in this investigation were MRSA (70%), followed by MSSA (30%). In all, 100% of *S. aureus* isolates were MDR (Figure 2).

Prevalence of resistant genes

The amplified *mec*A gene (167bp) was found in 61.6% (37/60) of the 60 *S. aureus* while the *van*A gene was not detected in any strain of *S. aureus*. *mec*A gene was found in 88.0% (37/42) of 42 MRSA (phenotypically confirmed MRSA) isolates and 100% (100%; 2/2) of VISA isolates (Figure 3). Neither of the 18 MSSA isolates had the *mec*A gene (Table 4).

Discussion

In the study, samples were collected from open heart surgery patients, in which 87 cultures were reported positive. The most prevalent bacteria found in the isolated samples were *S. aureus, K. pneumoniae,*

Table 3. Characteristics and frequency of S. aureus, MRSA, MSSA, VISA

Characteristics	*S. aureus* Frequency (%age)	MRSA Frequency (%age)	MSSA Frequency (%age)	VSSA Frequency (%age)	VISA Frequency (%age)
Clinical Specimen					
PUS	60 (100)	42 (100)	18 (100)	40 (100)	2 (100)
Gender					
Male	43 (71.6)	31 (73)	11 (61.1)	29 (72.5)	1 (50)
Female	17 (28.3)	11 (26.1)	7 (38.8)	11(27.5)	1 (50)
Age (years)					
<20	4 (6.6)	3 (7.1)	0 (0)	2 (5)	1 (50)
21-40	21 (35)	13 (30.9)	5 (27.7)	15 (37.5)	0 (0)
41-60	25 (41.6)	17 (40.4)	11 (61.1)	19 (47.5)	0 (0)
>60	10 (16.6)	9 (21.4)	2 (11.1)	6 (15)	1 (50)
Types of Patients					
Inpatients department (IPD)	38 (63.3)	32 (76.1)	13 (72.2)	31(77.5)	2 (100)
Outpatients department (OPD)	22 (36.6)	10 (23.8)	7 (43.7)	9 (27.5)	0

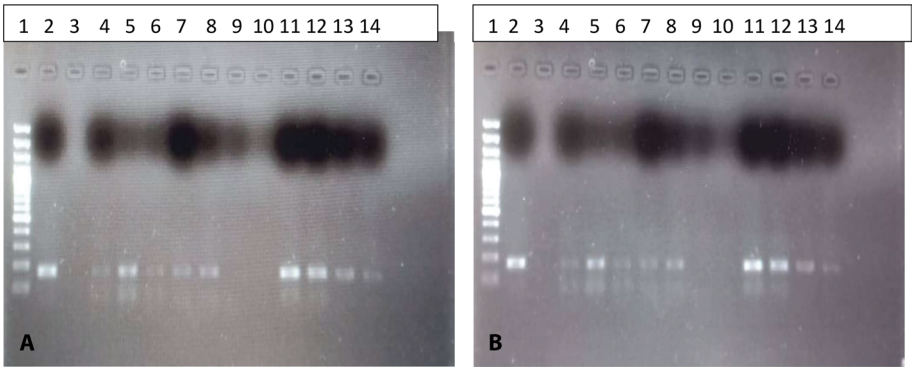

Figure 3. A and B: Represent gel images of PCR amplification of *mec*A (167bp. 100bp DNA ladder) genes. Lane 1 represent DNA ladder in the figure (A and B), while the other lane shown detection of genes.

E. coli and *Enterococcus* spp. *S. aureus* made about 68.9% of the isolates that were culture positive. Nearly 70% of the *S. aureus* strains were MRSA and every single one was MDR. 88% of the 42 MRSA isolates have the *mec*A gene, while no MRSA isolates possessed the *van*A gene. Similar bacterial growth patterns were seen in this investigation and observed in other clinical research in Pakistan (15). *S. aureus* was the most common bacteria found in this investigation; some earlier investigation shows that *E. coli* was the most common bacteria. This might be as a result of differences in research locations, sickness types, and clinical specimens.

The current study reports that male patients (73%) get MRSA more frequently than female patients. Similar results have been seen in Pakistan, where MRSA was more prevalent in males (54.7%) than females (45.3%) (26), while in another study, 65% of infected patients were men and 35% were women (27). In this study, *S. aureus* infections were more prevalent in the 41–60 age groups but in some report, infections were more prevalent in the 16–35 and 21–30 age groups (28).

In this study, most MRSA isolates were identified in pus specimens (100%). A similar result was shown in other investigations, where 70% of MRSA

Table 4. PCR Detection of Resistant *mec*A Gene (Number=60)

Organism	Number of Isolates	Detection of *mec*A Gene	
		Number	Percentage
S. aureus	60	37	61.6
MRSA	42	37	88.0
MSSA	18	0	0
VSSA	40	31	77.5
VISA	2	2	100

was found in pus specimens (29), compared to 17% in another research (26). The increased prevalence of MRSA in pus may result from *S. aureus's* opportunistic character and from their presence in the host's natural flora. In this investigation, 70% of isolates were found to be MRSA, whereas 30% were found to be MSSA. According to research from Lahore, Pakistan, MRSA isolates were found to be 100% MDR, whereas MSSA isolates were found to be 37.84% MDR and identified MDR-MRSA in 54% (28). These variations may be the result of antibiotic overuse, patient immunological state, geographic location, sample size, hospital management, quantity of samples and illness severity. For screening, majority of the research identifying at MRSA in Pakistan only used cefoxitin and oxacillin. Cefoxitin is thought of as a replacement marker and is a superior medication than oxacillin for detecting the *mec*A gene in MRSA. *mec*A gene detection or the company's PBP2a by cefoxitin product is regarded as the gold standard for confirming MRSA (13). These differences may have been caused by the length of the study period, the sample size, the number of study sites, the kind of sample, and the laboratory procedures utilized to evaluate the MRSA isolates.

In this study, the highest rates of MRSA resistance were seen for penicillin (100%), followed by cefoxitin (100%) gentamicin (95%), erythromycin (97%), Amikacin (83%), minocycline (83%), and ciprofloxacin (78%), while the lowest rates of resistance were seen for clindamycin (69%) and rifampicin (54%). In contrast, a study from Pakistan found that MRSA isolates had high resistance profiles against rifampicin (50%), clindamycin (30). Antibiotic uncontrolled usage, hospital infection control procedures, antibiotic self-medication, and strain nature might all contribute to variations in resistance profiles throughout the nation.

For the amplification of the *mec*A gene, a single pair of primers was used in the PCR process. Thirty seven *mec*A positive and 5 *mec*A negative MRSA strains were found out of a total of 42 isolates. The disc diffusion susceptibility technique validated the methicillin resistance of all 42 bacteria. This discovery could be the consequence of a false-negative PCR reaction, which can happen when the *mec*A gene has a point mutation or deletion or when there are inhibitors present. Studies have shown that several genes regulatory components, including the *mecI* and *mecR1* regulatory genes, fem factors (factors essential for methicillin resistance), are involved in the production or repression of *mec*A (31).

The *mec*A gene may be used in diagnostic laboratories to confirm the presence of MRSA strains, according to earlier research from Saudi Arabia (32), India (33), Australia (34), Japan (35) and the United States (36). Numerous studies contend that resistant isolates lack *mec*A, even though the identification of genes has long been considered as the gold standard among resistant isolates (37). Additionally, methicillin resistance to the drug was modest in isolates lacking the *mec*A gene (7). Although 74% of the isolates in our investigation tested positive for *mec*A, it is likely that the presence of this gene is important but not necessary for the emergence of resistance. Numerous internal variables could increase the emergence of resistance and stop the production of that genes. In a previous analysis from Nigeria, the gene product of PBP2, *mec*A, and the five primary SCC*mec* types were completely absent, but the isolates were nonetheless phenotypically resistant, indicating the likelihood of hyperproduction of β-lactamase (38). Another study hypothesizes that certain amino acid changes on protein binding cascades may play a role in the formation of MRSA. These changes can be brought about by identical or non-identical replacement of amino acids, as multilocus sequence typing showed in isolates of various sequence types (MLST) (39). According to this research, *mec*A is a pre-dominant but not exclusively responsible factor for conferring resistance in MRSA isolates; therefore, the methicillin resistance in this bacterial species may be attributed to the presence of extra intrinsic factors and pathways.

The results of this investigation indicate that MRSA cannot be detected using current diagnostic

methods (conventional and molecular). Combining these methods may help in properly identifying the prevalence and progress of MRSA as well as in directing antibiotic therapy. In our investigation, 4.7% of the MRSA isolates were VISA (vancomycin MIC), whereas the remaining 95.2% were VSSA (MIC). The results of this study are similar with those studied published in Mangalore, India (40). It is unclear how VISA resolves resistance. One theory states that the sequestration of glycopeptides (vancomycin and teicoplanin) from the accumulation of the peptidoglycan precursor acyl-D-alanyl-D-alanine dipeptide lowers the drug's penetration to its target region. Vancomycin is one among the antibiotics that can produce chromosomal anomalies when used at high dosages, however no gene or operon has been connected to VISA (41). None of the MRSA isolates in our investigation possessed the *van*A gene. Since the initial reports of VISA in 1997 (7) and VRSA in 2002 (42), Concern has been raised about *S. aureus* potential vancomycin resistance. Nevertheless, there are just a few cases of clinical infections brought on by VRSA on a global scale (43), whereas VISA-related illnesses are on the rise (44).

Conclusion

A high prevalence of MRSA among cardiac patients is a sign of a medical emergency since MDR strains can result in a variety of treatment failures, uncontrolled cardiac infections and deaths. Even if PCR-based detection techniques are superior to other traditional techniques, combining these approaches can provide accurate diagnostic results. It is believed that the widespread use of antibiotics for the past six decades has created resistant strains of bacteria due to misuse of antibiotics. This inefficient maintenance of health facilities has resulted in an increase in the deaths ratio associated with cardiac infections.

Author Contributions: Conceptualization, SK, KB and AU; methodology, KB, HI and SK; software, MB; SK and ZAS; validation, FUA, MS and ZAS; formal analysis, KB and MS; investigation, ZAS, KB and MB; resources, AU and KB; data curation, MS and HI; writing-original draft preparation, SK, and KB; writing-review and editing, AA, AU, and FUA; visualization, MB, HI; supervision, KB; project administration, AA and AU; funding acquisition, AA; All authors have read and agreed to the published version of the manuscript".

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