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Comparative Analysis of MRSA Isolation, Biofilm Formation, and Molecular Characteristics: Hospital-Acquired Versus Community-Acquired Strains

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Abstract: *The current study was to look into the molecular characterisation and biofilm development of MRSA in patients who visited the Khyber Teaching Hospital, Hayatabad Medical Complex, and Lady Reading Hospital in Peshawar. Antibiotic susceptibility tests identify Methicillin-resistant Staphylococcus aureus in confirmed isolates of S. aureus. PCR was used to identify a gene, which proved that the MRSA isolates were real. HA- and CA-MRSA were distinguished by SCC mec typing. The microtiter plate assay was employed to look for signs of biofilm formation. 133 (82.60%) of the 161 samples that were positive for S. aureus. Of them, 32 (19.87%) had MSSA isolates, 28 (17.39%) were culture-negative, and 101 (62.73%) were determined to carry MRSA. To confirm all positive isolates one more time, Mec A gene analysis was performed. Twenty-one (20.79%) of the eighty (79.20%) confirmed isolates were classified as undistinguishable MRSA isolates, twenty-six (32.5%) as HA-MRSA isolates, and fifty-five (67.5%) as CA-MRSA isolates.*

Key Words: MRSA, Community Associated MRSA, Hospital Associated MRSA, SCC mec Typing, Mec A gene

Introduction

In 1871, von Recklingausen made the first discovery that Staphylococcus aureus was present in human pyogenic lesions. Alexander Ogston called the plant Staphylococcus in 1880 because of the way the grapes resembled clusters (staphyle, which means a clump of grapes, and kokkos, which means berry). Ogston discovered non-pathogenic Staphylococci on skin surfaces. The Staphylococcus isolates from normal skin produced colonies that were golden yellow in colour, but most of the isolates from pyogenic lesions formed white colonies on solid media. These were subsequently named Staphylococcus aureus and Staphylococcus albus by Rosenbach in 1884. It was termed Staphylococcus albus before changing again to Staphylococcus epidermidis. According to Tyagi & Tyagi (2019), these isolates are coagulase-negative, non-pathogenic, and do not ferment mannitol.

The globally dispersed genus S. aureus has thirty unique species. One of the Staphylococci that is believed to be highly common and clinically significant is S. aureus (Zoubi et al., 2015). It has positive catalase and coagulase levels (Winn, 2006). Because S. aureus is immotile, when it is grown on nutrient agar, it produces enormous colonies that measure 60 mm, a phenomenon known as colony spreading (Kizaki et al., 2016).

S. aureus is a commensal bacteria that is found in the human microbiome. Because it is a resident of the gastrointestinal tract (GIT) and a part of the skin flora, it is easily spread from victim to victim by air or

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formites (Al-Zoubi et al., 2015). Colonisation is one of the primary causes of S. aureus infections. Many disease-causing variables allow this bacteria to enter host cells including epithelial cells (Al-Mebairik et al., 2016). Bhattacharya et al. (2015) report that one in three healthy individuals is asymptotically infected with this bacterium.

S. aureus is an important human pathogen that can cause septicemia, meningitis, toxic shock syndrome, endocarditis, and skin infections, according to Onaolapo et al. (2016). In hospitals as well as the general public, it is the primary cause of infections. Hospital infections can spread through treatment of any kind or through direct patient-provider interaction (Bhattacharya et al., 2015). S. aureus also plays a role in food contamination and toxic shock syndrome by releasing super antigens into the circulation (Todar, 2015). Medical procedures like surgery and transplants, as well as direct contact with medical personnel, can result in hospital-onset infections (Bhattacharya et al., 2015). Treatment for the infection may become challenging due to the rise of drug-resistant strains of S. aureus (Obajuluwa et al., 2016).

Onaolapo et al. (2016) state that S. aureus is an important human pathogen that causes septicemia, meningitis, toxic shock syndrome, endocarditis, and skin infections. It is the primary cause of infections in hospitals as well as the general population. According to Bhattacharya et al. (2015), hospital diseases can spread through direct patient-provider contact or any type of therapy. S. aureus also contributes to toxic shock syndrome and food poisoning by releasing super antigens into the circulation (Todar, 2015). According to Bhattacharya et al. (2015), medical procedures such as surgery and transplantation, as well as direct contact with medical personnel, might result in hospital start infections. Treatment for S. aureus infection may become challenging due to drug-resistant forms of the infection (Obajuluwa et al., 2016).

MRSA has been recognised as a spreading disease in the community and as an important pathogen in healthcare settings. Hospital-acquired MRSA infections are designated as HA-MRSA if patients were admitted to the hospital more than 48 hours prior to the onset of the illness (that is if the patient was not infected with MRSA at the time of admission but the infection and culture were discovered more than 48 hours after admittance). Community-acquired MRSA isolates have been recognised as a major pathogen in healthcare and community environments (Baldan et al., 2009). In 1965, there was a report of the first MRSA infection in Sydney (Fey et al., 2003; Fluit et al., 2001). In the United States, the first instance of infection due to community-associated MRSA was reported in 1980 (Ellis *et al.*, 2009).

Skin and soft tissue infections, as well as bacteremia, are mostly caused by CA-MRSA isolates. However, past studies have demonstrated that 25% of urinary tract infections are also brought on by CA-MRSA. Those who are not hospitalised or have not had medical treatments (such as dialysis, catheterization, or surgery) before being admitted to the hospital are the ones who get these infections. SCCmec type aids in differentiating between CA-MRSA and HA-MRSA because it might be difficult to determine the actual origin of each MRSA isolate (Parvez et al., 2018).

Throughout the community, the HA-MRSA are present and spread, particularly among adults. Furthermore, a number of studies have shown that MRSA clones are carriers of SCCmec type IV. Both intrinsically and phenotypically, CA-MRSA isolates differ from HA-MRSA isolates. These isolates resemble a few methicillin-sensitive S. aureus (MSSA) isolates in that they are susceptible to a range of anti-staphylococcal antibiotics (some are only resistant to -lactams), and they frequently produce Panton-Valentine Leukocidin (PVL), a poison that kills platelets and is a virulence factor of Staphylococcus (Ellis et al., 2009; Borriello et al., 2005).

Many MRSA strains are now identified as MDR, and the only antibiotic that can treat them is vancomycin. Even resistance to vancomycin is increasing at a low level. The most common causes of MRSA infections include prolonged hospital stays and excessive antibiotic use before admission. Medical personnel who work with these MRSA-infected patients are the main source of infection transmission; as carriers, they complicate therapy (Vysakh & Jeya, 2013).

Numerous virulence factors needed for pathogenesis are produced by S. aureus. Virulence determinants include hemolysin, biofilm, leukocidin, enzymes, and substances that disrupt the host immune system (Rusenova & Rusenove, 2017). All of these virulence factors are controlled by the accessory gene regulator (agr) system. In S. aureus, four agr types have been identified thus far (Pollitt et al., 2015).

Anton Valentine leukocidin (PVL) is another virulence factor that contributes to a variety of infections (Ahmad et al., 2020). It's a cytotoxin that

causes -barrel pores to develop. PVL contribute significantly to pathogenesis by specifically targeting the host's neutrophils (Niemann et al., 2018). PVL is expressed in just two to three per cent of S. aureus isolates. In addition to CA-MRSA, which is extremely common, it has been revealed that HA-MRSA also expresses PVL genes (Hu et al., 2015).

One of the biggest medical concerns is chronic infections, which are brought on by bacterial cells that form biofilm and develop resistance to antibiotic treatment. It is estimated that over 80% of human illnesses are caused by biofilms. According to Piechota et al. (2018), MRSA and MSSA bacteria generate biofilms, which are important virulence factors that affect their persistence in the host environment.

According to Gowrishankar et al. (2012), biofilms are a collection of different bacteria that grow on both biotic and abiotic surfaces and embed themselves in a polysaccharide matrix made of proteins and micromolecules. Biofilms are shielded against antimicrobial treatment via protein modulation, changes in gene expression, and metabolic activity (Anderson & Otoole, 2008). According to Neopane et al. (2018), S. aureus is a biofilm-forming bacterium that causes a range of illnesses, such as endocarditis, exotoxin syndrome, skin lesions, and tissue infections. Because this bacterium forms resistant biofilm structures, chronic infections continue and significantly increase morbidity and mortality in the human population (Todar, 2015).

Despite the fact that S. aureus has demonstrated enhanced susceptibility to chemotherapeutics in vitro, the therapy employed to treat infections caused by them frequently fails, leading to repeated clinical and persistent subclinical infections (Neopane et al., 2018). The development of bacteria as biofilm is the cause of these persistent and recurring S. aureus infections (Obajuluwa et al., 2016).

Methicillin-susceptible S. aureus clonal lineages have given rise to many MRSA sublineages by the acquisition of transposable elements called Staphylococcal cassette chromosomemec (SCCmec). The mechanical resistance is caused by a gene that is found in SCCmec. SCCmec is a part of the chromosome of MRSA. No other bacteria have been reported to contain SCCmec (Nagasundaram & Sistla, 2019). Numerous typing techniques, such as Staphylococcus cassette chromosome (SCCmec) typing, multilocus sequence typing (MLST), and pulse field gel electrophoresis (PFGE), have been developed for the purpose of characterising MRSA strains. Among them, SCC mec typing is among the quickest and least expensive methods (Mahomed et al., 2018).

Additionally, HA and CA-MRSA isolates have been distinguished by SCCmec type. The CA-MRSA isolates have SCC mec type IV and V, which do not contain any additional resistance genes save mec A, while the HA-MRSA strains have SCCmec types I, II, and III, which include genes that confer resistance to non- -lactam antibiotics (Nagasundaram & Sistla, 2019).

The structural composition and genetic organisation of SCC mec components are very diverse. So far, 12 SCC mec components with sizes ranging from 21 to 67 kbp have been described. Whereas CA-MRSA has SCC mec type (IV and V), HA-MRSA isolates have three SCC mec elements (I, II, and III). A junkyard (J) region, the mec gene complex, and the cassette chromosome recombinase (CCR) complex are present in every MRSA isolate. Mec A, Mec I, and Mec RI are regulatory genes that are part of the mec gene complex. Insertional inactivation genes have the potential to modify these regulatory genes. The deletion and insertion of the SCC mec elements at the 3′ end of the orfX require specific recombinases, which are encoded by the CCR gene complex The junkyard region of the cassette is not that important, although it contains extra antibiotic resistance components (Reichmann & Pinho, 2017).

The development of biofilms in MRSA-infected patients has been linked to a significant increase in both mortality and morbidity in humans. The capacity of resistant strains of MRSA to produce biofilm has been linked to a variety of illnesses, including septicemia, pneumonia, polyarthritis, necrotizing fasciitis, and endocarditis. To track the pattern of antibiotic resistance exhibited by MRSA in the area, several antibiotics had to be assessed. Furthermore, virulence genes aid in distinguishing isolates of CA-MRSA from HA-MRSA. The ability of CA-MRSA and HA-MRSA to form biofilms will aid in determining the level of resistance because strong biofilm formers exhibit significant antibiotic resistance.

Methodology

Sample Size

In the time period of two months, 161 different clinical samples were collected from different patients.

Sample Collection

Patients who visited various hospitals in Peshawar, such as the Hayatabad Medical Complex, Khyber

Teaching Hospital, and Lady Reading Hospital, provided samples for analysis. Every sample was obtained in compliance with CLSI criteria (2020).

Sample Processing

After collection, the samples were brought to the laboratory for analysis. Every sample was grown on blood agar and MSA. The plates were incubated at 37°C for the entire night (John et al., 2016).

Identification of *S. aureus*

According to John et al. (2016), S. aureus was identified by a variety of morphological and biochemical assays, such as Gramme staining, catalase, coagulase, and DNAse.

Morphological Identification of *S. aureus*

The samples were cultivated once more and incubated for the entire night at 37 °C. The next day, the colonies' appearance and features were examined.

Gram Staining of *S. aureus*

S. aureus was identified by gramme staining. Hans Christian Gramme developed this initial identification technique to differentiate between Gramme-positive and Gramme-negative bacteria.

Gram Staining Reagents

Primary stain, also known as Crystal Violet, Iodine, sometimes known as a mordant, ethanol or acetone (a decolourizer), and safranin dye were the reagents used in gramme staining (Bunter *et al.*, 2017).

Biochemical identification of *S. aureus*

Catalase Test

The catalase enzyme was found using the catalase test. Hydrogen peroxide is broken down by catalase into oxygen and water. A slide had two or three drops of hydrogen peroxide put into it. A sterile loop was used to assist in selecting and moving one colony to the slide. Positive outcomes are indicated by bubble development (Holt et al., 2013).

Coagulase Test

The Coagulase test is used to distinguish S. aureus from other species of Staphylococcus. The enzyme coagulase is responsible for converting fibrinogen into the sticky substance fibrin. After selection, the bacterial colonies were placed on the slide. Following careful mixing, human plasma was added to the culture and it was then incubated at 37 °C. A favourable outcome is indicated by the formation of a clot (Holt et al., 2013).

DNAse Test

On DNAse agar plates, the bacterial colonies were selected and cultivated. The plates were kept in a 37°C incubator all night. Following an overnight incubation period, 1 N HCL was used to wash the plates. Positive results are indicated by a unique zone that forms around the bacterial colonies (Kateete et al., 2010).

Antibiotic Susceptibility Testing of *S. aureus*

To assess the susceptibility of the S. aureus isolates, the Kirby Bauer disc diffusion technique was employed. The findings were interpreted in accordance with the antibiotic susceptibility testing recommendations published by CLSI (2020) (CLSI, 2020).

Preparation of McFarland Standard Solution

The McFarland equivalency standards are used in antibiotic

susceptibility testing around the world to verify the turbidity of bacterial suspension and keep the number of bacteria within a certain range. Barium chloride and sulphuric acid were used to make McFarland standard solution.

To analyze the absorbance, a 1cm light path spectrophotometer and balanced cuvette were used to test the correct density of the turbidity standard. The turbidity of the 0.5 McFarland was 1.5 10^8 CFU/mL which is comparable to the turbidity of bacterial solution (Collee *et al.*, 2006).

Antibiotic Discs Used

The antibiotics along with their concentrations are listed in Table 1.

Table 1

List of Antibiotics along with their Concentrations.

S. No	Antibiotics	Disc Concentration
1.	Cefoxitin	30 g
2.	Ciprofloxacin	5 g
3.	Gentamicin	10 g
4.	Chloramphenicol	30 g
5.	Erythromycin	15 g
6.	Clindamycin	2 g
7.	Linezolid	30 g
8.	Rifampicin	5 g
9.	Vancomycin	30 g
10.	Tetracycline	30 g
11.	Teicoplanin	30 g
12.	Fusidic acid	10 g
13.	Clarithromycin	15 g
14.	Oxacillin	1 g
15.	Moxifloxacin	5 g
16.	Ceftriaxone	30 g
17.	Meropenem	10 g
18.	Amikacin	30 g

Procedure of Susceptibility Testing

From a 24-hour fresh culture, two to three bacterial colonies were selected and injected in two millilitres of nutrient broth. The colonies were then incubated for six hours at 37 degrees Celsius, or until the turbidity matched that of the McFarland solution. Using a sterile swab, the inoculum was selected and smeared onto MHA plates. Using a sterilised syringe, various antibiotic discs were administered. Next, the plates were incubated at 37 °C for the entire night. Using a scale, the diameter of the antibiotic-formed zones was measured and compared to CLSI criteria (CLSI, 2020).

Biofilm Formation

Previous research has examined the ability of MRSA isolates to form biofilms. After mixing 250 L of MRSA broth with TSB, the mixture was placed on a microtiter plate and incubated for 94 hours at 37 °C. Saline was used to wash non-adhesive cells following the formation of biofilms. Crystal violet staining was then carried out. Following a series of rinses with distilled water to eliminate any leftover stain, ethanol was added to each well and the cells were allowed to air dry at room temperature. The absorbance at 570 nm was then measured using an ELISA reader. The medium of culture served as a control. Three categories were used to categorise the isolates: strong (OD 570 0.5), medium (OD 570 0.2), and weak.

DNA Extraction

The S. aureus pure colonies were suspended in approximately 300 L of TE (Tris-HCL EDTA) buffer with a pH of 8. To extract the supernatant, the cell culture was heated at 100 °C and then centrifuged for five minutes at 15,000 rpm. The supernatant was collected in a different tube. After that, 95% cooled ethanol was added, and it was centrifuged for 5 minutes at 15,000 rpm after being kept at -20 °C for 20 minutes. Prior to PCR amplification, the DNA template was combined with 50 L of nuclease-free water and kept at -20 °C. A nanodrop spectrophotometer was used to measure the concentration and purity of the extracted DNA. DNA purity was measured at 260/280 OD. The extracted DNA's integrity was

Polymerase Chain Reaction

PCR Master Mix Preparation

After lyophilized primers were mixed with PCR water to create a stock solution, 10 L of the stock solution was used to create the working solution.

Detection of *mecA* gene by PCR

The mechanical gene was amplified by means of the conventional PCR method. Table 2 lists the primers that were utilised. To produce the reaction mixture, 12.5 L of Bioshop master mix, 1 L of each primer

(forward and reverse), 8.5 L of nuclease-free water, and 2 L of DNA were added to each reaction, for a total of 25 L per reaction. The PCR procedure was carried out using the programme listed in Table 3.4 (Nasution et al., 2018).

Table 2

mecA Gene Primers.

S.No	Target gene	Oligonucleotide Sequence(5'-3')	Specificity	Product Size (bp)
1	*mecA*	**F:** TGCTATCCACCCTCAAACAGG **R**:AACGTTGTAACCACCCCAAGA	*mecA*	284

F= Forward R=Reverse

Table 3

PCR Conditions for Amplification of mecA Gene.

Gene	Program					
	Initial denaturation	Cycles	Denaturation	Annealing	Extension	Final extension
mecA	94°C for 5 minutes.	35	94°C for 1 minute.	59.4°C for 1 minute.	72°C for 1 minute.	72°C for 10 minutes.

Gel Electrophoresis

Agarose was dissolved in 1 X Tris-acetate EDTA solution to create a 1% agarose gel. After heating the solution for two minutes in a microwave oven, 5 L of Ethidium bromide (1 g/ml) was added to the agarose. Ethidium bromide was added right away, and the mixture was then transferred into a gel tray with a seven-well comb. The electrophoresis equipment was activated and the gel coated after loading (Bhattacharya et al., 2019).

SCCmec Typing

By utilising PCR, SCC mec cassettes were found. CIF2 (explicit for SCC mec type 1), DCS (explicit for SCC mec types I, II, IV, and VI), KDP (explicit for SCC mec type II), RIF4 and RIF5 (explicit for SCC mec types III), MEC I (explicit for type II), and SCC mec VJI were all identified using gene-specific primers.

Multiplex PCR was carried out for amplification using a thermal cycler (Milheirico, 2007: Oliveira & Lancaster, 2002). Primers for SCC*mec* typing are listed in Table 3.5.

Table 4

Primers for Scc mec Elements.

S.No	Target Gene	Oligonucleotide Sequence (5'-3')	Specificity	Product Size (bp)	Reference
1	*CIF2*	**F:**TTCGAGTTGCTGATGAAGAAGG **R:**ATTTACCACAAGGACTACCAGC	SCC*mec* type I	495	Milheirico*et al.*, 2007
2	*KDP*	**F:**AATCATCTGCCATTGGTGATGC **R:**CGAATGAAGTGAAAGAAAGTG G	SCC*mec* type II	284	Milheirico*et al.*, 2007
3	*MEC1*	**F:**ATCAAGACTTGCATTCAGGC **R:**GCGGTTTCAATTCACTTGTC	*SCCmec*type II, III	209	Milheirico*et al.*, 2007
4	*DCS*	**F:** CATCCTATGATAGCTTGGTC **R:**CTAAATCATAGCCATGACCG	*Sccmec*type I II IV VI	342	Milheirico*et al.*, 2007
5	*RIF4*	**F:**GTGATTGTTCGAGATATGTGG **R:** CGCTTTATCTGTATCTATCGC	*Sccmec*type III	243	Milheirico*et al.*, 2007
6	*RIF5*	**F:**TTCTTAAGTACACGCTGAATCG **R:**GTCACAGTAATTCCATCAATGC	*Sccmec*type IV	414	Milheirico*et al.*, 2007
7	*SCCmec VJ1*	**F:** TTCTCCATTCTTGTTCATCC **R:**AGAGACTACTGACTTAAGTGG	*Sccmec*type V	377	Milheirico*et al.*, 2007

F=Forward R=Reverse

Results

Total Number of Samples Processed

161 samples in total were gathered and examined. 133 of the 161 samples had S. aureus confirmed. 28 (17.39%) of the total samples obtained were culture-negative, and 101 (75.93%) isolates of methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-sensitive Staphylococcus aureus (MSSA) were found. Total culture positive and negative samples are displayed in Table 4.1.

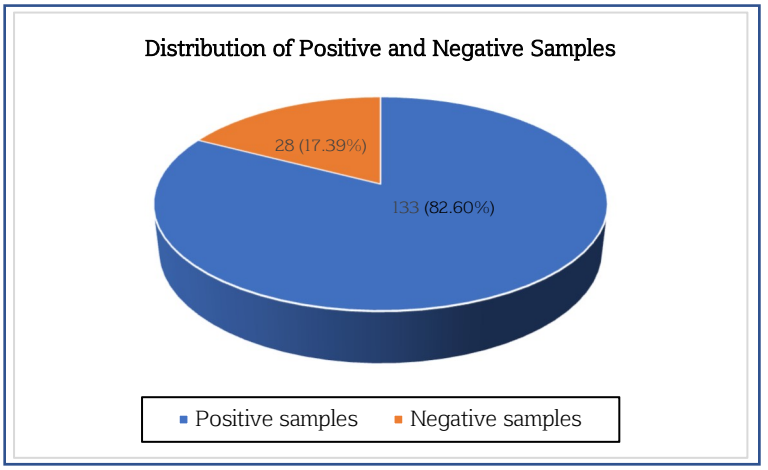
Table 5

Total Culture Positive and Negative Samples.

Total No. of Samples	Culture Positive Samples	Culture Negative Samples
161	133 (82.60%)	28 (17.39%)

Figure 1

Distribution of positive and negative samples.



Gender-Based Ratio

Samples that were taken, 54 (40.60%) came from patients who were female, and 79 (59.40%) came from patients who were male.

Table 6

The percentage of male and female patients infected with S. aureus.

Gender	No. of Samples Collected	Percentage
Female	54	40.60 %
Male	79	59.40 %
Total	133	100 %

Morphological Identification of *S. aureus*

Colonies of S. aureus isolates took on a golden hue when cultivated on blood agar plates. On mannitol salt agar plates, it developed vivid yellow-coloured colonies.

Microscopic Identification of *S. aureus*

Under a microscope, *S. aureus* colonies had violet, circular, or cocci-shaped colonies that looked like bunches of grapes.

Biochemical Identification of *S. aureus*

The results of different biochemical assays that were performed to identify *S. aureus* are listed in Table 4.3. All the isolates 133(82.60%) were positive for oxidase, catalase, DNase and Coagulase test.

Table 7

The Results of Different Biochemical Tests.

S. No	Biochemical Test	Result
1	Oxidase	Positive
2	Catalase	Positive
3	DNAse	Positive
4	Coagulase	Positive

Antibiotic Susceptibility Testing of *S. aureus*

After 24 hrs of incubation, the plates were examined and the diameter of the zones was measured and interpreted according to CLSI guidelines. The results of all the 133 isolates indicated that 101 (62.73%) were Methicillin Resistant *Staphylococcus aureus* (MRSA) and 32 (19.87%) isolates were Methicillin Sensitive *Staphylococcus aureus* (MSSA). Table 4.4. shows the antibiogram of all 133 isolates of *S. aureus*.

Table 8

Antibiogram of *S. aureus* isolates.

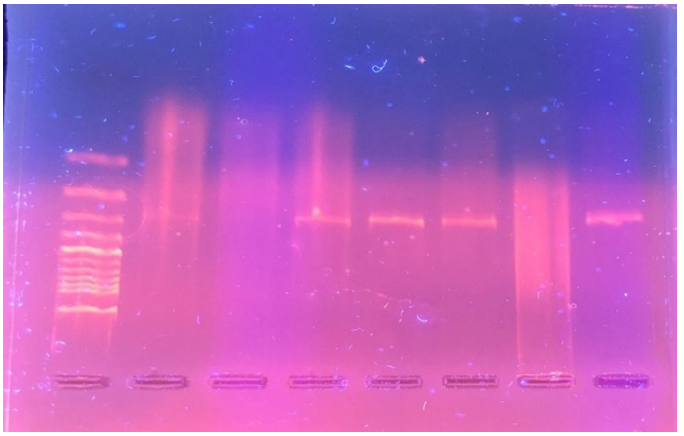
S. No	Antibiotics Used	Abbreviation	Sensitive	Intermediate	Resistant
			n (%)	n (%)	n (%)
1.	Cefoxitin	FOX	26 (19.54)	6 (4.51)	101 (75.93)
2.	Ciprofloxacin	CIP	20 (15.03)	9 (6.76)	104 (78.19)
3.	Gentamicin	CN	16 (12.03)	11 (8.27)	106 (79.69)
4.	Chloramphenicol	CHL	25 (18.76)	6 (4.51)	102 (76.69)
5.	Erythromycin	E	26 (19.54)	4 (3.0)	103 (77.44)
6.	Clindamycin	DA	17 (12.78)	8 (6.01)	108 (81.20)
7.	Linezolid	LZD	105 (781.94)	3(2.25)	25 (18.76)
8.	Rifampicin	RIF	22 (16.54)	7 (5.26)	104 (78.19)
9.	Vancomycin	VA	109 (81.95)	3 (2.25)	21 (15.78)
10.	Tetracycline	TET	20 (15.03)	6 (4.51)	107 (80.45)
11.	Teicoplanin	TEC	30 (22.55)	1 (0.75)	102 (76.69)
12.	Fusidic acid	FD	68 (51.12)	3 (2.25)	62 (46.61)
13.	Clarithromycin	CLR	26 (19.54)	4 (3.0)	103 (77.44)
14.	Oxacillin	OX	31 (23.30)	1 (0.75)	101 (75.93)
15.	Meropenem	MEM	9 (6.766)	-	121 (90.97)
16.	Amikacin	AK	21 (15.78)	6 (4.51)	106 (76.69)
17.	Moxifloxacin	MXF	10 (7.51)	11 (8.27)	112 (84.21)

MecA Gene Detection

Mec A gene is responsible for resistance to methicillin in gram-positive bacteria, especially *S. aureus*. Out of a total of 133 (82.60%) *S. aureus* isolates *mec*A gene was detected in 101 (75.93%) isolates.

Figure 2

Mec a gene Detected in MRSA.



SCC Mec Typing

Eighty (79.20%) of the 101 (75.93%) MRSA isolates were categorised as CA and HA-MRSA. Of the 80 confirmed isolates (79.20%), 26 (32.5%) were identified as CA-MRSA and 54 (67.5%) as HA-MRSA. Multiple SCC mec types were present in both groups, making it unable to identify the remaining 21 (20.79%) MRSA isolates. SCC mec I and IV were found in 2 (9.52%) of the 21 (20.79%) undistinguishable MRSA isolates. Fourteen isolates (66.66%) had SCC mec I and III detected, while five isolates (23.80%) had SCC mec I, III, and IV detected.

SCC mec IV was found in 10 (38.46%) and SCC mecV in 16 (61.53%) of the 26 (32.5%) CA-MRSA isolates, while SCC mec I was found in 8 (14.81%) of the 54 (67.5%) HA-MRSA isolates. 42 (77.77%) isolates had SCC mec III found, while 4 (7.40%) isolates had SCC mec II detected.

Figure 3

Detection of SCC Mec Elements

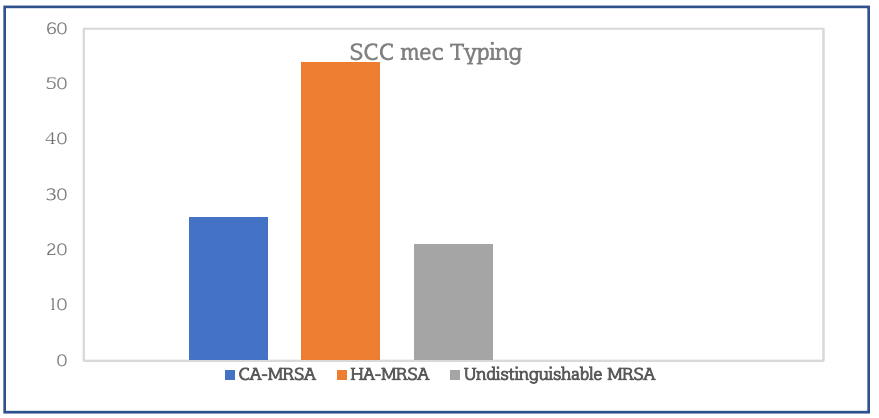


Figure 5

KDP Gene Detected in MRSA

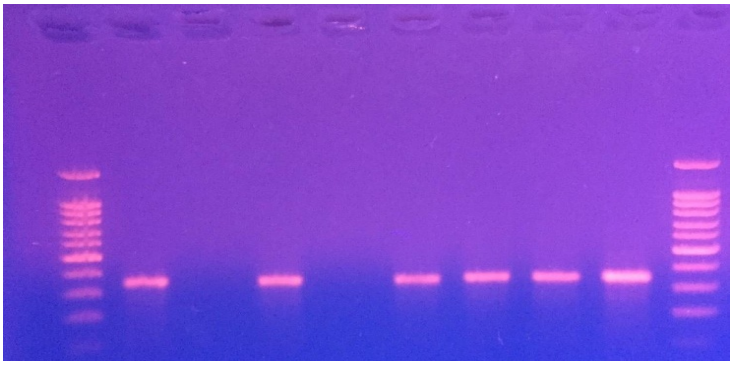


Figure 6

DCS Gene Detected in MRSA.

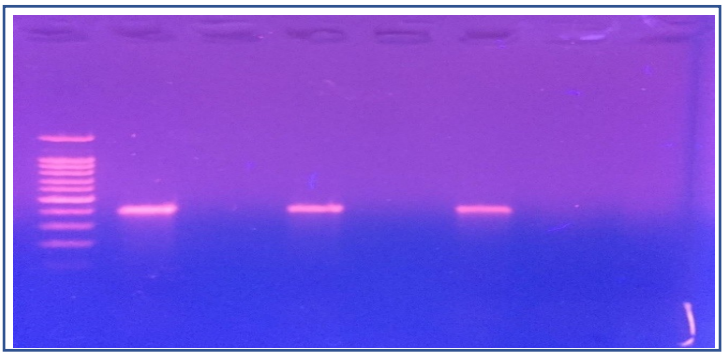
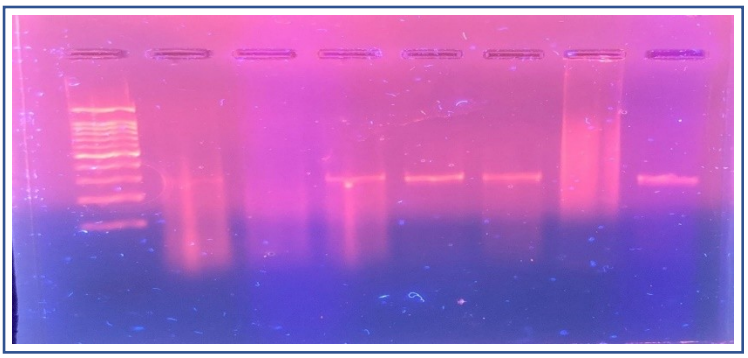


Figure 7

RIF4 gene Detected in MRSA.



Detection of Biofilm Formation

Twenty-seven (88.46%) of the twenty-six (25.74%) CA-MRSA isolates were strong biofilm formers, two (7.69%) were moderate, and one (3.84%) was weak. Nine (16.66%), four (7.40%), and 41 (75.92%) of the 54 (53.46%) HA-MRSA isolates were moderate biofilm formers, while the remaining isolates were strong biofilm formers.

Fig. 4.9 shows the biofilm formation of *S. aureus*.

Figure 8

Detection of Biofilm Formation of CA-MRSA S. Aureus.

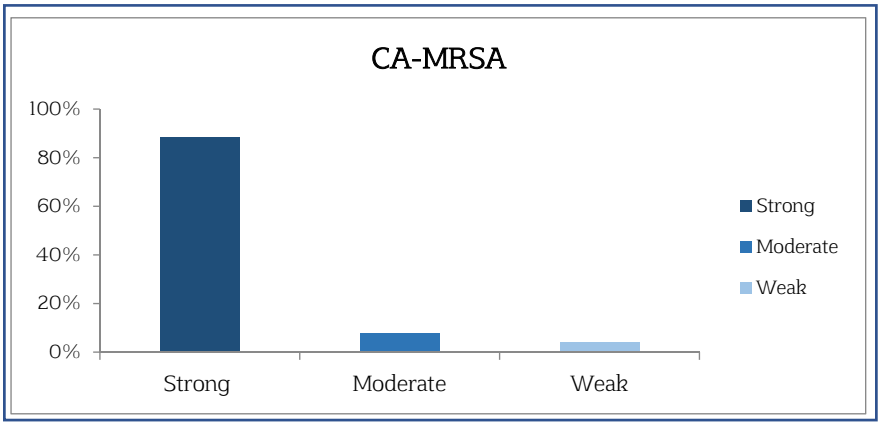


Figure 9

Detection of Biofilm Formation of HA-MRSA S. Aureus.

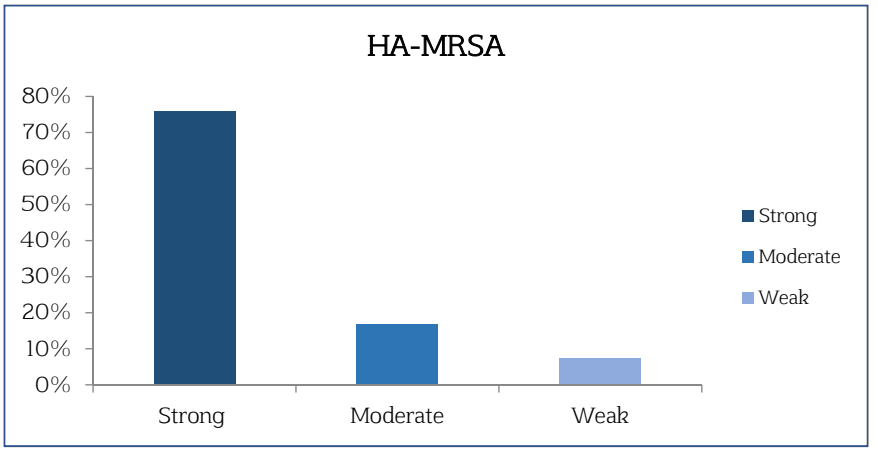
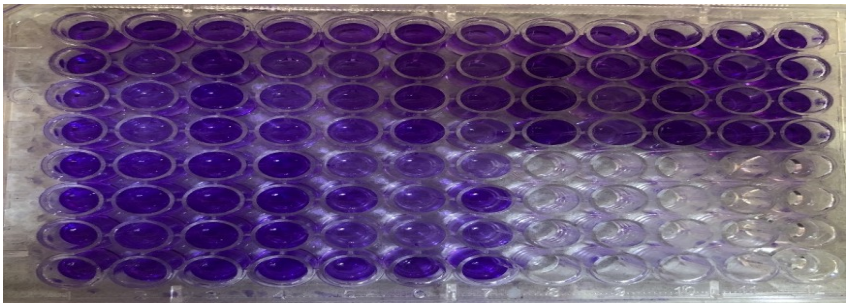


Figure 10

Biofilm formation of CA and HA-MRSA.



Discussion

A common bacteria in the human population, Staphylococcus aureus is carried by a large number of asymptomatic individuals. Some of its strains have evolved into MRSA, which can lead to infections that are potentially fatal. High rates of death, morbidity, and financial ramifications for hospitals worldwide are associated with S. aureus infections. Due to S. aureus's adaptability, variety of virulence factors, and increased level of antibiotic resistance, treating these infections can be difficult for medical professionals (Zhen et al., 2020; Chen et al., 2020). This study aimed to evaluate the pattern of antibiotic resistance, biofilm development, and molecular characterization of MRSA from both community and hospital settings. Antibiotic abuse may be the cause of the substantial level of antibiotic resistance shown in the data.

Many people have Staphylococcus aureus, a bacteria that is widely distributed throughout the human population, without causing any symptoms.82.60% of the samples in the current investigation had S. aureus detected in them. According to Parvez et al. (2018), 81.53% of the S. aureus in Bangladesh's samples were isolated, which is in line with their findings. This aligns with the conclusions of a recent Ethiopian study carried out by Gizachew et al (2015). Methicillin-resistant Staphylococcus aureus (MRSA) made up 62.73% of the isolates in the current investigation, whereas methicillin-sensitive Staphylococcus aureus (MSSA) made up 19.87% of the isolates.

On the other hand, high frequencies of MRSA—80 and 90%, respectively—were found in studies carried out in Peru and Colombia by Blanco et al. (2009) and Jimenez et al. (2012). Similarly, 61.9% of the MRSA isolates in Iran were reported by Havaei et al. (2014) and Moshtagheian et al. (2018). Similar results from Taiwan and Nigeria were reported by Wang et al. (2015) and Malley et al. (2015).

According to the most recent results, 81.95 per cent of the isolates were susceptible to vancomycin, whereas 15.78 per cent had resistance to the antibiotic. Similar results were published by Garoy et al. (2019), which corroborate these findings. A total of 101 (75.93%) of the isolates in the current investigation were cefoxitin (FOX) resistant. according to Mbim et al.'s study (2017). Over the past ten years, MRSA cases have progressively climbed worldwide. resistant to methicillin Antibiotic-resistant Staphylococcus aureus is well recognised to thrive when antibiotics are present. Antibiotics are administered differently depending on local guidelines in different regions, which can be explained by the variable resistance pattern of MRSA. The increased usage of first-line antibiotics has made microorganisms more resistant to them. Latif & Sohail (2018).

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The *mec*A gene is responsible for conferring methicillin resistance. *Mec* A gene is found on *SCC mec*, which is detected by PCR Asghar, (2014). In the present study, the *mec*A gene was detected in 101 (75.93%) isolates as previously reported by Asghar, (2014). Similarly, in a study conducted by Jafari (2019), 68% of *mec*A gene was detected. In another study conducted by Ghanwate *et al.*, (2016), 77.77% of the *mec*A gene was detected.

A total of 26 (32.5%) of the CA-MRSA and 54 (67.5%) of the HA-MRSA isolates were found in the current investigation. Similarly, a total of 15 (35.7%) of the CA-MRSA and 27 (64.3%) of the HA-MRSA were found in a study by Mbim et al. (2017). Comparably, 22 (57.90%) and 16 (42.10%) of the CA-MRSA and HA-MRSA in Bangladesh were reported by Parvez et al. (2018). However, because of their high prevalence rate, Sohail & Latif's (2018) investigation found that the isolation rates of HA-MRSA and CA-MRSA were 43% and 57%, respectively. According to the current results, SCC mec types IV and V were found in 10 (38.46%) and 16 (61.53%) isolates, respectively, although SCC mec type IV was found in a study by Funaki et al. (2019).

Due to its resistance to a variety of medicines, S. aureus which forms biofilms is significant from a therapeutic standpoint. According to Neopane et al.

(2018), this resistance is typically greater in S. aureus which forms biofilms than in S. aureus which does not. In contrast to a study by Hosseini et al. (2020), where 52.9% of the isolates were strong, 45% were moderate, and 22.5% of the total isolates were weak biofilm formers, the current findings showed that 23 (88.46%) of the CA-MRSA isolates were strong, 2 (7.69%) moderate, and 1 (3.84%) weak biofilm formers. In contrast, 58 (84%) of the isolates in research by Tabandeh et al. (2021) were strong biofilm formers, 17 (80%) were moderate, and 7 (58.3%) were weak.

Conclusion

MRSA is a pathogenic bacteria that is a global public health concern that needs to be properly monitored and treated in community and hospital settings. This study demonstrates that the prevalence of MRSA is higher than that of MSSA in this region. The substantial level of antibiotic resistance caused by localised antibiotic misuse was revealed by the results. It was also found that most of the isolates of S. aureus were susceptible to linezolid and vancomycin, while cefoxitin resistance was present. Therefore, it's crucial to often examine the antibiotic profile. In the location, these antibiotics may be the recommended treatment for treating MRSA. The results show that HA-MRSA is more common in this area than CA-MRSA and that CA-MRSA has been demonstrated to be a more potent biofilm producer than HA-MRSA.

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