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Research Article

Molecular Detection of *mec*-A Gene and Antimicrobial Profiling of Clinically Associated *Staphylococcus aureus*

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Abstract

Staphylococcus aureus is accountable for a high number of infections in individuals within healthcare settings. Studies have shown that hospital-acquired methicillin-resistant *Staphylococcus aureus* (HA-MRSA) has become a pandemic in healthcare and hospital facilities with substantial variation in the infection rate within different geographical locations. The aim of the study was molecular identification of MRSA in clinical samples and antibiogram profiling of *S. aureus* isolates. A total of 50 samples (from blood, pus and urine) were selected after initial identification and were confirmed through conventional biochemical tests. Molecular identification and detection of MRSA strains were done via primer-specific 16s RNA polymerase chain reaction (PCR) targeting the *mec*-A gene. Antibiotic susceptibility was performed against eight routine antibiotics. Among the 50 samples, 11 were positive for *mec*-A gene. Antibiotic profiling revealed that 84% of the samples were sensitive to Sulfamethoxazole, 64% were sensitive to Cefoxitin, 56% were sensitive to Vancomycin and 52% were sensitive to Tazobactam. Conversely, 78% of the isolates were Erythromycin resistant, 62% were Oxacillin resistant, 50% were Clindamycin resistant and 48% were resistant to Ciprofloxacin. Most of the bacterial strains were resistant to more than one antibiotic thus implying the high resistance rate of *S. aureus* against routinely administered antimicrobials. Furthermore, the study suggests that molecular methods such as PCR targeting the *mec*- A gene can be effective for the detection of MRSA.

Keywords: Antibiotic Susceptibility; Antimicrobial Resistance; Molecular Detection; PCR; *S. aureus*

Introduction

Staphylococcus aureus continues to be one of the most prevalent human pathogens causing a variety of diseases ranging from mild soft tissue and skin infections to infective necrotizing pneumonia, bacteremia, septicemia osteomyelitis and endocarditis leading to debilitating and life-threatening outcomes [1-3]. Methicillin-Resistant *Staphylococcus aureus* (MRSA) is the most frequent drug-resistant strain which is developed when methicillin-susceptible *S. aureus* (MSSA) acquires *mec A* gene, encoding the penicillin-binding protein 2′ or 2a (PBP2′ or PBP2a) following its integration within staphylococcal cassette chromosome *mec* (SCC*mec*) i.e., the large chromosomal element [4]. Thus, the *mec* A gene is a key molecular marker used for the detection of Methicillin-Resistant Staphylococcus aureus (MRSA). Molecular techniques, such as polymerase chain reaction (PCR) assays targeting the *mec* A gene, offer rapid and accurate identification of MRSA within hours serving as a gold standard for MRSA identification [5]. The irrational and inappropriate use of antibiotics has led *S. aureus* strains to develop antimicrobial resistance [6]. MRSA colonization increases the risk of septicemia and bacteremia leading to serious clinical

complications [7,8]. The high antimicrobial resistance exhibited by *S. aureus* makes it a formidable threat in hospital and community settings as it is widely associated with increased morbidity and mortality rates [9,10].

Due to its genetic diversity and multi-resistance potential, *S. aureus* is ranked as one of the most crucial nosocomial pathogens [11,12]. While in recent years, MRSA stains were responsible for 25-50% of clinically associated infections [13]. Healthy humans and animals carry *S. aureus* asymptomatically as colonizers residing in the nares and mucous membranes [14,15], however, it can act as an opportunistic pathogen once it breaches the innate host immune defenses by invading the bloodstream and intestinal tissues [16]. The increased resistance towards the anti-infective treatment not only makes it difficult to deal with this sturdy pathogen but also leads to a high economic burden including factors such as expensive treatment due to high complexity, prolongation of illness, readmission because of surgical site infections and increased duration of stay at the hospital [17]. Therefore, the global prevalence of MRSA is at a progressive increase with substantial regional variation. According to the European Antimicrobial Resistance Surveillance System (EARSS), hospital-acquired MRSA (HA-MRSA) in acute and long-term healthcare settings range between 1%-24% with a significant intercountry and intra-country variation [18]. WHO has reported that the mortality rate is higher for MRSA-infected patients compared to non-MRSA-infected patients [19]. Thus, MRSA imposes a greater threat due to its adaptation capabilities in various environments, its ability to cause a vast array of life-threatening infections and its high virulence capacity thereby contributing to the failure of standard empirical therapy [20,21].

Local factors associated with the dissemination of *S. aureus* infections can be identified and quantified through effective medical surveillance systems following the initiation of steps to abate the spread of pathogens. The present study involves antimicrobial profiling and molecular detection of MRSA strains from clinical sources blood samples (25), pus samples (14) and urine samples (11) in order to gain insights regarding the current antimicrobial trends and prevalence of MRSA in clinical settings.

Materials and Methods

Microorganisms

Fifty clinical isolates potentially suspected to be *S. aureus* (provided by HOPES lab Karachi, Pakistan) were collected from clinical sources (blood, pus and urine) after initial screening. The samples were aseptically streaked on Mannitol Salt Agar (MSA) and stored at 4°C.

Biochemical testing

The suspected *S. aureus* isolates were subjected to microscopic examination and biochemical testing (Coagulase test, catalase test, DNase test) to confirm the identity of the isolated strains.

Antibiotic profiling

The clinically isolated strains were tested using the Kirby-Bauer disk diffusion technique according to CLSI standards [22] against the following antibiotics; Erythromycin (15µg), Ciprofloxacin (5µg), Vancomycin (30µg), Clindamycin (2µg), Oxacillin (1µg), Cefoxitin (30µg), Tazobactam (110µg) and Sulfamethoxazole (25µg). The procedure involved sterile wire loops to carefully transfer colonies of 2mm in diameter from an 18-hour culture. These colonies were immersed in Mueller Hinton broth (Oxoid) contained in sterile bottles and incubated for 5 hours. Gradually, sterile saline was added to the broth to achieve a turbidity that could be compared to the 0.5 McFarland standard, corresponding approximately to 1.0 $\times 10^8$ cfu/ml. The bacterial suspension was then spread uniformly onto the surface of a Mueller-Hinton agar plate. Antimicrobial disks of specific concentrations were placed on the agar, and the plates were incubated for 24 hours at 37°C. The zones of inhibitions were measured in millimeters using a zone reader on subsequent day. All test were performed in triplicates for each sample.

DNA extraction

The DNA was extracted from fifty isolated samples. GJC® bacterial DNA purification kits were used to purify DNA from bacterial cells [23]. Bacterial cells were inoculated in 5ml tryptone soy broth (TSB) for 24 hours. 1.5ml of the sample from the TSB broth was aseptically removed and centrifuged at 14,000 rpm for 5 minutes and the supernatant was discarded followed by the addition of 400µL of buffer (S1) and the cells were resuspended in a pal-

let. 20 μL of Proteinase K enzyme was added into the tube and was thoroughly mixed *via* vortexing and the tubes were incubated at 56°C to achieve full cell lysis. 200 μL of buffer (S2) was added to the sample, mixed *via* pulse vortex for 30 seconds and incubation of the sample at 70°C for 10 minutes in a water bath. 200 μL of laboratory grade ethanol (96-100%) was added to the sample and thoroughly mixed *via* vortex for 30 seconds. 600 μL of the mixture was then pipetted into the GJC® mini spin column followed by centrifugation at 8000rpm for 1 minute. The GJC® mini spin column was then placed in a fresh 2ml collection tube following the addition of 600ul of buffer solution (S3) and centrifugation at 10,000 rpm for 3 minutes, after which the flow through was discarded.

The mini spin column was placed in a clean 2ml collection tube followed by addition of 600 μL of buffer (S4) and centrifugation at 14,000 rpm for 3 minutes, the flow through was discarded. The GJC® spin column was then placed in a fresh 2ml collection tube followed by addition of elution buffer (EB) and centrifuged at 10,000 rpm for a minute to elute out the DNA.

Gel electrophoresis

To evaluate the presence or absence of the *mec-A* gene, the 270bp gene fragment was amplified *via* real-time PCR. Specific primers were selected and purchased from Synbio technologies (USA) for amplification of the *mec- A* gene segment. Reverse and forward primers were used described by Kumar., *et al*. 2016 [24] (Table 1).

Agarose gel, 2% (Merck) measuring 8 cm was prepared using a TAE (Tris-acetate-EDTA) buffer solution as the conductive medium for gel electrophoresis. The gel electrophoresis was carried out at a constant voltage of 80 volts for a duration of 40 minutes. Each well was loaded with 8 μL of the sample, consisting of DNA fragments. Bromophenol blue loading dye was added to the samples to track the progress of electrophoresis and aid in visualization. After the electrophoresis run, the gel was observed under a UV transilluminator to visualize the separated DNA bands. DNA ladder (100bp) was used for size determination. The cycling parameters for the PCR are described in Table 2.

Table 1: Oligonucleotide primers for 16S rRNA gene amplification.

Primer	Product Size	Sequence	Reference
*mec*A-F	270 bp	5'AACGATTGTGACACGATAGCC3'	Kumar., *et al*. (2016) [24]
*mec*A-R		5'GGGATCATAGCGTCATTATC3'	

Results and Discussion

Fifty clinical isolates were tested in the laboratory through various biochemical, cultural, staining and molecular methods to confirm the identity of organisms while 11 samples were positive for the *mec A* gene. The PCR assay successfully amplified the 16S rRNA gene target from the template DNA (270bp fragment). PCR results for *S. aureus* are depicted in Figure 1. Antimicrobial profiling of all 50 isolates was performed using the Kirby-Baur disc diffusion technique (Figure 2) and the susceptibility patterns were determined according to CLSI guidelines (Table 3). Among the 50 *S. aureus* isolates 78% were Erythromycin resistant, 62% were Oxacillin resistant, 50% were Clindamycin resistant and 48% were resistant to Ciprofloxacin. Conversely, 52% of the samples were sensitive to Tazobactam while 56%, 64% and 84% were susceptible to Vancomycin, Cefoxitin and Sulfamethoxazole respectively. All the *mec- A* positive strains were resistant to more than one antibiotic. The resistance profile for the isolates was generated according to susceptibility test interpretive criteria by CLSI, 2016 (Figure 3).

Methicillin resistance is a vital factor to consider while mediating antibiotic treatment within clinical settings. The *mec A* gene serves as a gold standard to identify methicillin resistance as well as being a distinguishing factor between the MSSA and MRSA strains [25,26]. Antibiotic resistance to pathogens has been a worldwide threat to public health both in clinical and community settings imposing an elevated risk of infection among children and elderly individuals [27,28]. In clinical settings, MRSA exhibits a number of virulence factors including toxins (leukocidins and haemolysins), enzymes promoting tissue invasion (hyaluronidase) and surface factors capable of evading the immune system (capsule and protein A). Notably, high mortality rates (approximately 20%) are associated with invasive MRSA infections most frequently in healthcare settings usually occurring in individuals having predisposing risk factors such as those who are immunocompromised or who underwent surgery [29].

Table 2: Cycling parameters for Polymerase chain reaction (PCR).

Phase	Temperature	Duration
Initial denaturation	94 °C	5 minutes
30 cycles	94 °C 50 °C 72 °C	1 minute 2 minutes 2 minutes
Final extension	72 °C	10 minutes

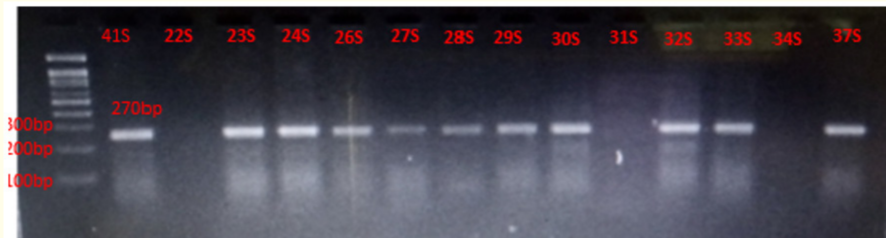

Figure 1: A representative gel electrophoresis image of PCR on UV transilluminator for detection of mec A gene with mec A-F and mec A-R primers depicting results of fourteen different samples showing amplified products. Negative control without amplicon and positive control with the expected bands were not shown. 22S and 34S showed negative results.

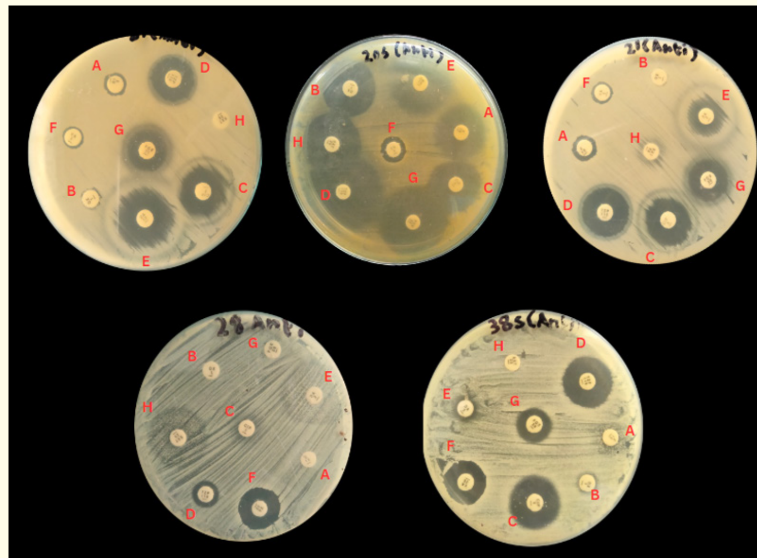

Figure 2: Zones of inhibition of various antibiotics against *S. aureus* isolates. Where; (A) Erythromycin, (B) Oxacillin, (C) Ciprofloxacin, (D) Tazobactam, (E) Clindamycin, (F) Vancomycin, (G) Cefoxitin and (H) Sulfamethoxazole.

Antimicrobial Agent	Antibiotic Susceptibility Patterns								
	Resistant			Intermediate			Sensitive		
	Blood (n = 25)	Urine (n = 11)	Pus (n = 14)	Blood (n = 25)	Urine (n = 11)	Pus (n = 14)	Blood (n = 25)	Urine (n = 11)	Pus (n = 14)
Erythromycin	19	03	06	05	06	09	01	-	01
Oxacillin	16	03	03	-	01	-	09	05	14
Ciprofloxacin	13	04	04	04	02	04	08	03	08
Tazobactam	09	04	04	-	-	-	16	05	12
Clindamycin	14	02	02	08	2	08	03	05	07
Vancomycin	18	07	07	-	-	-	07	02	07
Cefoxitin	16	04	04	-	-	-	09	05	13
Sulfamethoxazole	13	03	03	-	-	-	12	06	14

Table 3: Antibiotic susceptibility chart of 50 *S. aureus* isolates on the basis of inhibition zones.

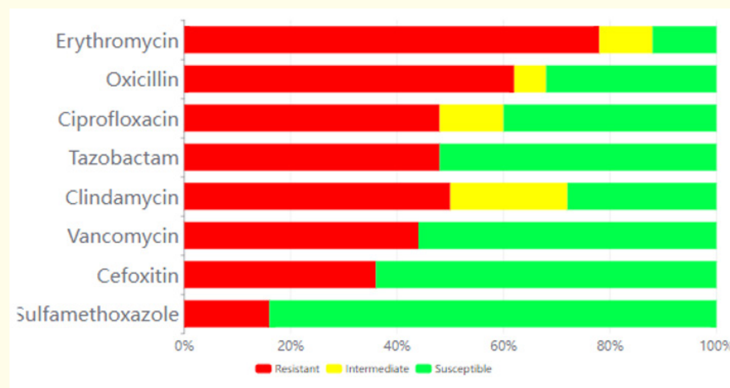

Figure 3: Antibiotic resistance profile of eight antibiotics against fifty S. aureus isolates represented as a stacked bar chart.

In recent years the profuse use of antibiotics has undoubtedly accelerated the process of resistance development in *S. aureus* by the acquisition of multiple resistance genes which has made the species particularly difficult to treat. The susceptibility trend of most used antibiotics, as evaluated in the undertaken study demonstrated that most strains were susceptible to Sulfamethoxazole, Cefoxitin and Vancomycin while exhibiting resistance against Erythromycin and Oxacillin [30,31]. Sulfamethoxazole had the highest percentage susceptibility while Erythromycin was the least effective with the lowest susceptibility.

The limitation of the undertaken study is its relatively small sample size therefore large-scale studies are further required for the evaluation of the opted molecular methods and to analyze the trends of antibiotic resistance.

Conclusion

The study shows that resistant strains of *S. aureus* are frequently prevalent in hospital settings infecting individuals, causing bacteremia and uremia leading to life-threatening outcomes. Molecular detection of *mec- A* gene is an effective method to differentiate between MRSA and MSSA strains but requires appropriate DNA extraction. 16S rRNA gene was targeted due to its conserved and variable regions, allowing universal primers for diverse species offering phylogenetic insights with a large sequence database. Primer specificity and extensive use make it reliable for microbial identification. Other rRNA genes have specific applications but lack 16S versatility and established role. Moreover, most of the isolates showed resistance to multiple drugs which is quite concerning

thereby indicating the need for effective infection control mechanisms and preventive measures against the development of virulent strains.

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