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# PCR based detection of *mecA* gene of *Staphylococcus aureus* isolated from surgical wound patients in Quetta

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## Abstract

*Staphylococcus aureus* is an acquired hospital infection and which has been shown to develop resistance against several antibiotics. Polymerase Chain Reaction (PCR) was performed to categorize bacterial antibiotic-resistant. The present study aimed to examine *S. aureus* in pus samples through using Polymerase Chain Reaction and study the resistance of the bacterial isolates against certain antibiotics. A total of (n=100) pus samples were obtained from surgical wound patients through sterile cotton swabs. The samples were immediately cultured, and positive samples were tested for gram staining, biochemical test, and antibiotic susceptibility. Our study showed that 58(58%) of the surgical patients were found positive for *S.aureus* and 42 (42%) were showed negative. This current study represents the *S.aureus* bimolecular identification and screening test of strain methicillin-resistant bacteria from the pus of surgical wounds of different hospitals from Quetta Balochistan. From hundred samples of 58 isolates of *Staphylococcus aureus* were confirmed through biochemical and PCR-based techniques. This current study reveals the *S.aureus* resistance variability against different antibiotics.

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## Introduction

*Staphylococcus aureus* is known as a human pathogenic as well as a commensal bacterium. Around 30% of *S.aureus* colonized the human population. In human beings, *Staphylococcus* is responsible for widespread diseases, such as mild skin infections to life-threatening conditions including pneumonia, endocarditis, toxic shock, and septicemia (Wertheim *et al.,* 2005). Methicillin-Resistant *Staphylococcus aureus (*MRSA) is a common human pathogenic bacterial strain that causes community-acquired and nosocomial infections (Shopsin and Kreiswirth, 2001).

The occurrence of MSRA has become more common in many regions of the world, resulting in serious infections in hospitals that impose a serious burden on medical and socio-economic charges and cause a major illness as well as death (Sajith Khan *et al.,* 2012).

After the appearance of MRSA in the United Kingdom in 1961 (Jevons, 1961), MRSA is a hospital bug that spreads all over the world (Timothi, 2004). MRSA is now responsible for 30% or more of all severe infections (Borriello *et al.*, 2005). Prolong-stay in the hospital, misuse of antibiotics, lack of consciousness, over-the-counter antibiotics availability, etc., are the potential causes for MRSA emergence (Anupurba *et al.*, 2003).

The cause of MRSA resistance is because of the Penicillin-binding protein (PBP2a) regulated by mecA genes (Andre *et al.,* 2008), placed on the *Staphylococcal* cassette chromosome (SCC) (Katayama and Hiramatsu, 2000), a huge genetic mobile diverges in size and genetic composition between MRSA strains (Ito *et al.*, 2001). Different forms of SCC mec cases are generally considered by methods of PCR. The resistant tool comprises alterations or faults transported by transformation on mecA gene, which results in antibiotic resistance. Additionally, antibiotic resistance genes might exist in the cassette, making it resistant to several antibiotics (Katayama and Hiramatsu, 2000). Finding of *mec*A gene by Polymerase chain reaction (PCR) is generally recommended essential for methicillin resistance because these genes are highly conserved among *Staphylococca*l species (Jonas, 2002).

## Materials and methods

### *Study design*

The current study was conducted at CASVAB University of Balochistan Quetta during the period of March 2019 to December 2019. A total of 100 samples of surgical wound patient pus were collected from (BMC) Bolan Medical Complex Quetta. Wound samples were collected aseptically through a sterile cotton swab and transported to the laboratory in cold chain conditions.

### *Identification of S.aureus*

The samples were cultured on Brain heart infusion (BHI) broth and incubated at 37℃. After incubation, the bacterial culture was inoculated on Mannitol Salt (MSA) agar plates and then plates were incubated at 37 ℃ (Biswas *et al.*, 2015). The pure isolated colonies were picked up for the biochemical characterization and identification was performed through gram staining, oxidase, catalase, and MRVP, Coagulase and citrate utilization test (Bannerman, 2003).

### *Antimicrobial susceptibility testing*

Antimicrobial susceptibility was done by the method of disk diffusion and the protocol followed of Clinical and Laboratory Standards Institute (CLSI). The culture of *S. aureus* (0.5 McFarland) was made and spread to one of Muller Hinton agar (MHA) plates and then antibiotic discs were placed at equal distances and incubated at 37℃. The *S. aureus* isolates were checked against Tetracycline, Levofloxacin, Erythromycin, Oxacillin, Vancomycin, Imipenem, Gentamycin, Ciprofloxacin, Chloramphenicol, and Cefoxitin (Wayne, 2002).

### *Molecular identification*

The isolated *S.aureus* culture was inoculated onto BHI broth and incubated at 37℃. DNA was isolated from overnight bacterial culture through the boiling method (Ali *et al.,* 2014). Isolated DNA was stored at

-20.

*PCR amplification for 16SrRNA*

PCR was performed for positive isolated samples of *S.aureus*. The positive isolated samples were characterized by the thermal cycler PCR. The primer following sequence F-5′-GTAGGTGGCAAGCGTTATCC-3′ R-5′-CGCACATCAGCGTCAG-3′ were intended to consent to the strengthening of 288 bp fragment of 16SrRNA gene. PCR reaction mixture was prepared as follows 20ul of reaction mixture containing 10ul of master mix, 3ul of PCR water,1ul of each primer ( forward and reversed) and 5ul of template DNA.

The amplification was carried out in a Thermal Cycler (Eppendorf, Germany) with an initial denaturation at 94 ℃ for 5 minutes, denaturation at 94℃ for 45 seconds, annealing at 58℃ for 45 seconds, extension 72 ℃ for 1 min, final extension at 72℃ for 10 min followed by 37 cycles.

*PCR amplification for mecA gene*

The primer following sequence F- 5′- AAAATC GATGGTAAA GGT TGG C 3′ R- 3′ AGTTCTGCAGTACCGGATTTGC 5′ were designed to allow the amplification of 533bp fragment of *mecA* gene. PCR reaction mixture for the amplification of *mec A* gene as follow 20ul reaction containing 10ul of PCR master mix, 3ul of PCR water, 1ul of each primer (forward, reversed) and 5ul of template DNA.

The amplification was carried out in a thermal cycler with an initial denaturation at 94℃ for 30 seconds, annealing at 55℃ for 30 seconds, extension phase at 72℃ for 1 minute, final extension step at 72℃ for 5 minutes followed by 40 cycles.

**Results**

A total of (n=100) samples of pus were taken from wound surgical patients, out of which 58 (58%) samples were found to be positive for *S.aureus* and 42 (42%) samples found to be negative.

**Table 1.** Gender wise distribution of pus patients.

| Gender | No of patients | Percentage |
|---|---|---|
| Male | 35 | 60% |
| Female | 23 | 40% |

The gender-based distribution revealed that male patients 35(60%) were highly infected than female patients 23(40%), as shown in Table 1. The positive samples of *S.aureus* on selective medium Mannitol salt agar (MSA) showed yellowish golden color colonies appearance as shown in Fig. 1. The pure isolated colonies were identified through gram staining, catalase, oxidase, coagulase, MRVP, citrate utilization and gelatin liquefication test, as shown in Table 2.

**Table 2.** Biochemical tests for *Staphylococcus. aureus.*

| Catalase | Oxidase | Coagulase | Indole | MR | VP | Citrate | Gelatin |
|---|---|---|---|---|---|---|---|
| +ve | -ve | +ve | -ve | -ve | +ve | +ve | +ve |

The antibiotic susceptibility testing showed that the following antibiotic is highly resistant such as Chloramphenicol 12(20%), Tetracycline 10(17%), Cefoxitin 8(13%), and Vancomycin 6 (10.3%) and generally, Gentamycin 9(15%) was sensitive as shown in Table 3. The PCR was used for the amplification of *mecA*gene. All positive isolates were phenotypically identified and gave a single band at 533bp mecA gene, as shown in Fig. 2. PCR was used for the amplification of 16SrRNA. The positive isolates harbor 228 bp fragment of 16SrRNA gene, as shown in Fig. 3.

**Discussion**

*Staphylococcus aureus* causes worldwide serious health issues in many hospitals and it's becoming resistant to many antibiotics. In our study wound, pus

samples were obtained from 100 patients. *Staphylococcus aureus* isolates on selective MSA media were identified as *Staphylococcus aureus* showed the prevalence of 58 (58%) positive for *S.aureus* and 42 (42%) were found negative that is in compliance with the findings of (Humaryanto *et al.*, 2020). Our study revealed the gender-wise distribution showed that male patients 35 (60%) were highly infected than female patients 23(40%). The differences in isolation of MRSA rates between studies might be due to the difference in time periods and locations of the studies, variance in hygienic conditions maintained in different hospitals, implementation of infection regulator program, and misuse of antibiotics, that may range from hospital to hospital (Bissong*et al.*,2016).

**Table 3.** Antibiotic susceptibility testing of *Staphylococcus aureus*.

| | Antibiotics | Resistant (%) | Sensitive (%) |
|---|---|---|---|
| 1 | Tetracycline | 10(17%) | 2(3.4%) |
| 2 | Levofloxaicn | 7(12%) | 5(8.6%) |
| 3 | Erythromycin | 5(8.6%) | 8(13%) |
| 4 | Oxacillin | 4(6.8%) | 7(12%) |
| 5 | Chloramphenicol | 12(20%) | 2(3.4%) |
| 6 | Vancomycin | 6(10.3%) | 9(15%) |
| 7 | Imipenem | 2(3.4%) | 7(12%) |
| 8 | Gentamycin | 2(3.4%) | 9(15%) |
| 9 | Ciprofloxacin | 2(3.4%) | 6(10.3%) |
| 10 | Cefoxitin | 8(13%) | 3(5.1%) |

Antibiotic use has increased dramatically over 50 years, resulting in the occurrence of bacterial strain resistance and the spread of genes resistance between harmful microbes (Karthy*et al.*, 2009).

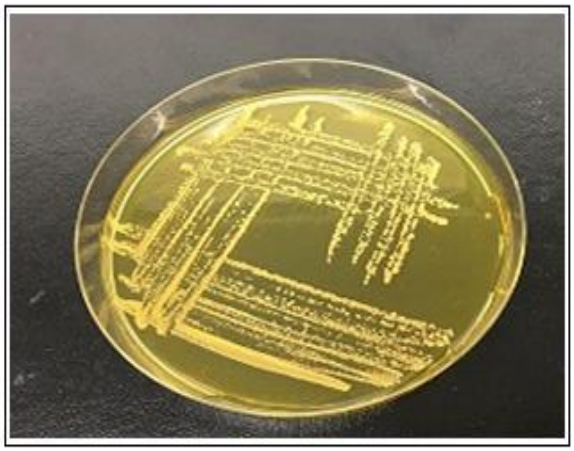

**Fig. 1.** *Staphylococcus aureus* colonies on Mannitol salt agar.

In our study, the sensitivity pattern of *S.aureus* to the following antibiotics; Tetracycline, Levofloxacin, Erythromycin, Oxacillin, Chloramphenicol, Vancomycin, Imipenem, Gentamycin, Ciprofloxacin, and Cefoxitin was performed.

The antibiotic susceptibility testing showed that the following antibiotic is highly resistant such as Chloramphenicol 12 (20%), Tetracycline 10 (17%),Cefoxitin 8 (13%), and Vancomycin 6 (10.3%) and generally Gentamycin 9 (15%) was sensitive Similar outcome was informed by (Sisirak*et al.*, 2010). Results revealed multiple drug resistance (MDR) of antibiotics against Vancomycin, Gentamicin, Chloramphenicol, Erythromycin, Cloxacillin, and Tetracycline.

The occurrence of *Staphylococcus aureus* Methicillin Resistant (MRSA) in Pakistan range from 2 to 61%. MRSA is becoming increasingly common in number in large cities of Pakistan.

All the isolates in our study were also confirmed by PCR. The PCR was used for the amplification of *mecA* gene. All positive isolates were phenotypically identified and given a single band at 533bp mecA gene. Many researchers worked on phenotypic and genotypic identification of staphylococcus aureus by using 16S rRNA gene (Bakeet *et al.*, 2014).

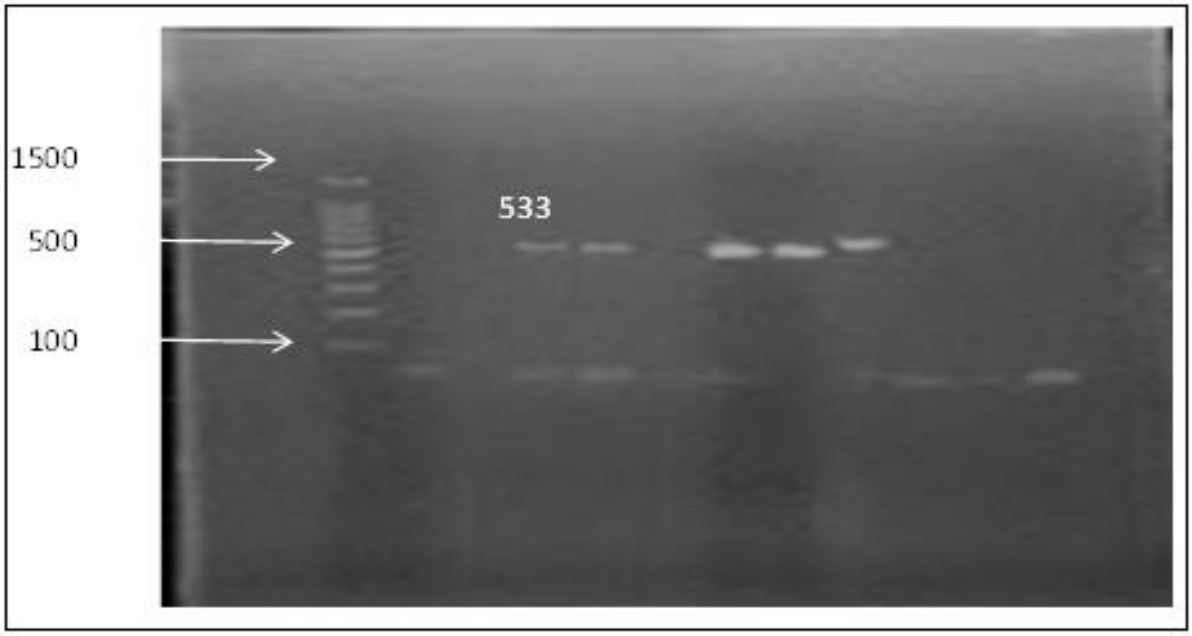


**Fig. 2.** PCR assay shown positive amplification of mec A gene,Lane M represents the 100bp DNA ladder, lane 1 negative control lane 2-10 positive isolates.

Similar findings also reported Molecular Identification for *Staphylococcus aureus* 16S rRNA gene isolated from Wounds and Burns. Several other studies have also confirmed 16Sr RNA method by PCR for reliability and quick detection of *Staphylococcus aureus* (Al–Alak and Kadhim Qassim, 2016).

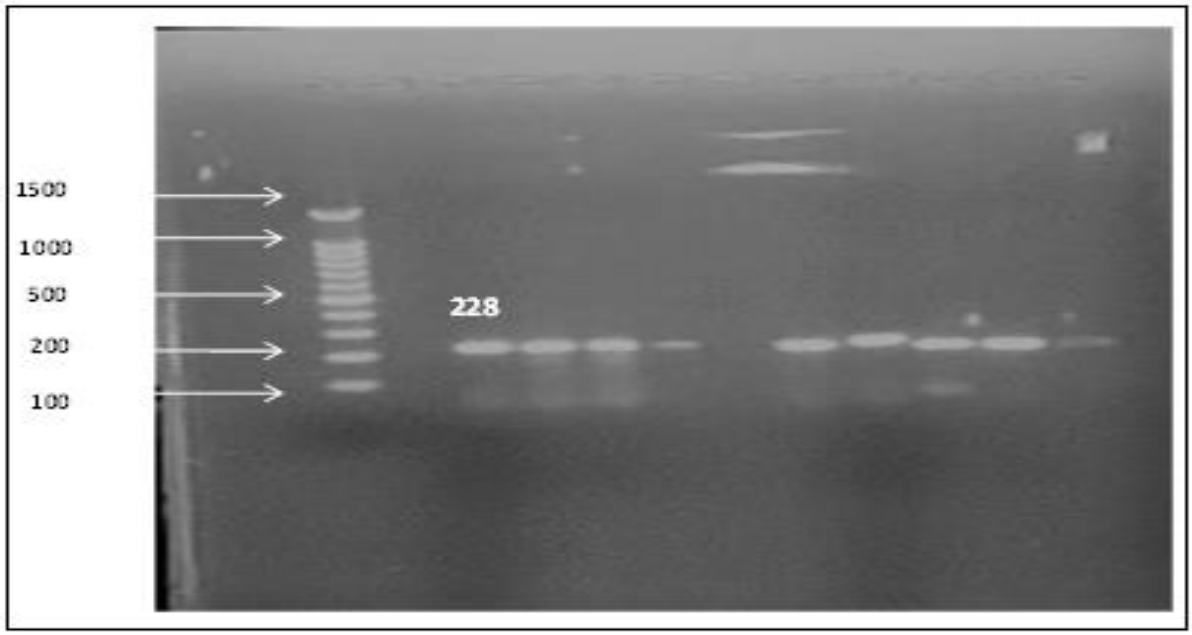


**Fig. 3.** PCR assay showed the amplification of 16SrRNA.M lane represents molecular ladder of 100bp, lane 1 is the negative control and lane 2-10 is the positive isolates of 16SrRNA.

**Conclusion**

This current study represents the bimolecular identification of *Staphylococcus aureus* and the test for screening of methicillin-resistant strain of surgical wounds from hospitals of Quetta Balochistan. From 100 samples of 58 isolates of *Staphylococcus aureus*

confirmed through biochemical and PCR-based techniques. Our study revealed the resistance variability of *S. aureus* against different antibiotics. To conclude, PCR was found to be a speedy and correct method for the discovery of MRSA infection linked to conservative approaches; meanwhile, the period taken for PCR assay is much less, prompt treatment can be initiated in view of medical and socio-economic costs.

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