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Isolation, Detection and Molecular Characterization of *Staphylococcus aureus* from Postoperative Infections

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Authors' contributions

This work was carried out in collaboration among all authors. Author RMJ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RVG and TL managed the analyses of the study. Author RVG managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Post operative infections that occur after surgical procedures can cause a lot of complications like sepsis, organ failure or even death. These are the third most commonly reported healthcare associated infection. The most common cause of wound infection regardless of procedure performed remains gram-positive cocci which comprise more than 50% of all infections. Specifically, *Staphylococcus aureus* and coagulase-negative staphylococci are the most frequent organisms isolated from a wound infection. There has been an increasing incidence of MRSA strains reported in hospitals across the globe. The main aim of our study is isolation, detection and molecular characterization of *Staphylococcus aureus* from postoperative infections. Samples were collected from post operative patients with infected wounds. The area around the wound was

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cleaned. Exudates were collected from the wound with a sterile swab stick. The samples were inoculated on different solid culture mediums and the plates were incubated in the presence of oxygen at 37°C overnight. There were many standard procedures done in which tube coagulase was taken as the main criteria. Antibiotic susceptibility testing was done by Kirby Bauer method following Clinical and Laboratory Standards Institute (CLSI) guidelines using commercially available cefoxitin (30 µg) disc (HiMedia) and the results were compared with *Staphylococcus aureus* ATCC 25923 and MRSA ATCC 43300 control strains. The MRSA strains were identified and detection of Mec A gene that codes for methicillin resistance is done using PCR technique.

Keywords: Staphylococcus aureus; MRSA; infections; hospital associated infections; bacteria.

1. INTRODUCTION

Post operative infections that occur after surgical procedures can cause a lot of complications like sepsis, organ failure or even death. These are the third most commonly reported healthcare associated infection. The most common cause of wound infection regardless of procedure performed remains gram-positive cocci which comprise more than 50% of all infections. Specifically, *Staphylococcus aureus* and coagulase-negative staphylococci are the most frequent organisms isolated from a wound infection. There has been an increasing incidence of MRSA strains reported in hospitals across the globe and there appears to be an association between nasal and skin colonization with this organism and subsequent postoperative infection. *Staphylococcus aureus* is a gram-positive bacteria that is cocci-shaped which appears to be in clustered form and are characterized as "grape-like" [1]. These bacteria spread through direct contact with an infected person, through the use of a contaminated object, or through inhalation of infected droplets dispersed by sneezing or coughing [2]. Skin infections are common, but the bacteria can spread and infect distant organs through the bloodstream. S.aureus infection rates were 1.7% within 60 days and 2.3% within 180 days of the procedure, representing 15.0% of the major infections [3]. Postoperative infections convey significantly increased clinical risks and health-care costs [4]. *Staphylococcus aureus* (S. aureus) infections, other gram-positive organisms including Clostridium difficile, gram-negative organisms such as pseudomonas, Escherichia. Coli, enterococci, and fungal infections are significant due to their increasing rates, antibiotic resistance [5].

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a large cause of hospital-acquired (nosocomial) infections in humans [6]. MRSA-infections respond poorly to beta-lactam therapy, and MRSA's resistance to multiple antimicrobials, including aminoglycosides, macrolides, clindamycin, and tetracyclines, is common [7]. It may be difficult to classify MRSA by routine susceptibility testing; therefore clinicopathology laboratories should implement techniques for the detection of MRSA [8]. As the number of hospital and community-acquired human MRSA infections is rising, it is likely that animal MRSA infections will also become more common [9].

Molecular approaches are of paramount importance in showing clonality and spreading patterns of S. aureus strains in hospital settings; however, variation exists about their effectiveness, cost and general applicability [10]. Polymerase chain reaction (PCR) based approach of characterising Staphylococcal cassettes mec types and the determination of sequence polymorphism in the variable X region of the Staphylococcal protein a (spa) has been shown to be relatively less expensive, easier, less time consuming and clearly discriminatory compared to other approaches, such as multi-locus sequence typing (MLST) and pulse field gel electrophoresis (PFGE) [11]. We present S. aureus by means of spa sequence typing and SCCmec genotyping. So the main aim of our study is isolation, detection and molecular characterisation of S. Aureus from postoperative infections [12,13].

2. MATERIALS AND METHODS

A total number of 25 samples were collected from post operative patients with infected wounds. The area around the wound was cleaned with 70% ethyl alcohol followed by normal saline and exudates were collected from the wound with a sterile swab stick soaked in normal saline or sometimes by aspirating with a sterile syringe and needle.

The samples were inoculated on Nutrient agar, Blood agar, MacConkey's. The plates were incubated aerobically at 37°C overnight. The

colonies suggestive of *Staphylococcus aureus* were identified by standard procedures (Gram staining, catalase test, slide coagulase and tube coagulase test, phosphatase test etc.,) Tube coagulase was taken as the main criteria for identification of *Staphylococcus aureus*.

Antibiotic susceptibility testing was done by Kirby Bauer method following Clinical and Laboratory Standards Institute (CLSI) guidelines using commercially available cefoxitin (30µg) disc (HiMedia) and the results were compared with *Staphylococcus aureus* ATCC 25923 and MRSA ATCC 43300 control strains. All *Staphylococcus aureus* strains isolated were screened for MRSA by detection of resistance to Cefoxitin disc (zone of inhibition was ≤21 mm) following the CLSI guidelines.

2.1 Detection of mecA Gene by PCR Technique

MRSA strain recognition was achieved by the discovery of the mecA gene in both S. aureus varieties that use PCR analysis. Tests showed that 45.1 per cent (126/279) of isolates from Staphylococci bore mecA genes. The standard PCR assay was performed using the DNA amplification instrument Master cycler gradient (Eppendorf, Germany) to identify MRSA strains. Cellular DNA was obtained from Staphylococci colonies grown overnight on blood agar plates using DNA Extraction Kit in accordance with manufacturer's instructions. The mecA- specific primer pairs used for amplification of 533 base pair (bp) fragment are Forward, 5′-AAA ATC GAT GGT GAA GGT TGG-3′, and Reverse, 5′-A GTT CTG GAG TAC CGG ATTTGC-3′. A volume of 1 µL of prepared DNA (0.5 µg) was added to a final volume of 25 µL PCR mixture containing 10 µL of 2× Master Mix (Ampliqon, Denmark), including 1× PCR buffer, 1.5 mmol/L MgCl2, 0.15 mmol/L dNTP, and 1.25 IU Taq DNA polymerase, (Ampliqon Co., Denmark), 0.7 µL of 0.8 µmol/L each primer and 12.6 µL of sterile distilled water. The thermal cycling protocol for PCR comprised 95°C for 3 min, followed by 33 cycles of 94°C for 1 min, 53°C for 30 s and 72°C for 1 min, with a final extension at 72°C for 6 min. The amplified products were visualized by electrophoresis in 2% agarose gels stained with ethidium bromide.

3. RESULTS AND DISCUSSION

Out of 25 samples collected, *staphylococcus aureus* was isolated from 9 samples (36%). The presence of *Staphylococcus aureus* confirmed using gram staining, which showed gram positive cocci in clusters (Fig. 1), cultural characteristics, golden yellow colonies on nutrient agar (Fig. 2) and detection of enzyme coagulase by both slide test and tube test. Out of 9 samples positive for *Staphylococcus aureus* 2 were found to be MRSA (Methicillin *Resistant Staphylococcus aureus*). Methicillin Resistant *Staphylococcus aureus* (MRSA) was shown by their resistance to cefoxitin antibiotic. A number of research studies have shown that the use of Cefoxitin in Staphylococci has a higher sensitivity and specificity than other compounds traditionally recommended for the detection of mecA resistance. Cefoxitin is an effective regulatory inducer of mecA. In *Staphylococcus aureus* (MRSA) it is recommended for the detection of methicillin resistance when using disk diffusion testing. MRSA strain was confirmed by the detection of the mecA gene using PCR analysis as shown in Fig. 3.

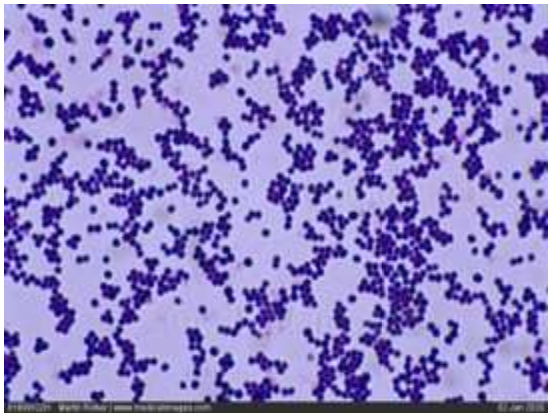

Fig. 1. Gram staining showing Gram positive cocci in clusters

S. aureus is an important pathogen that causes serious infections both in hospitals and Community. These resistant microbial strains have become one of the major concerns of the Clinicians, Microbiologists and Public Health officials. MRSA is especially troublesome in hospitals, where patients with open wounds, invasive devices and weakened immune systems are at greater risk of nosocomial infection. Resistance to antimicrobial agents developed by microorganisms has created a major problem in the treatment of not only serious and life threatening infections in Hospitals but also common infections at the Community level. The common complications following all operative procedures are surgical site infections. Preoperative care, the theatre sterility,

postoperative care, overcrowding, and the type of surgery are some of the factors which determine the surgical site infections. Contamination from the external environment is the most probable reason for the wound infection. This study was focused on finding out of a simple, economic and more accessible method to identify MRSA which is resistant to many antibiotics and it is very difficult to eradicate from patients as well as carriers.

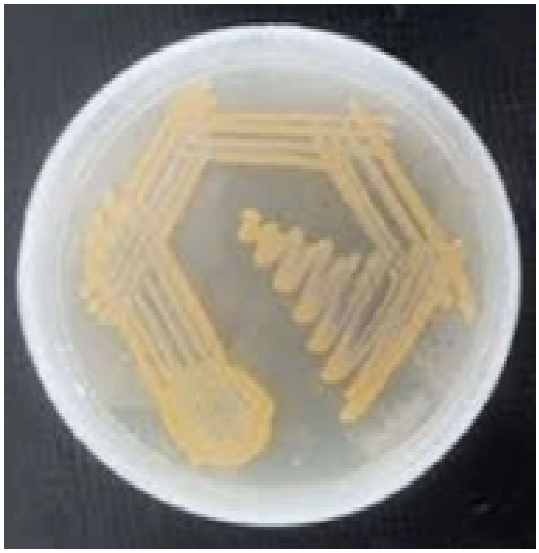

Fig. 2. Golden yellow colonies of *S. aureus* in Nutrient agar

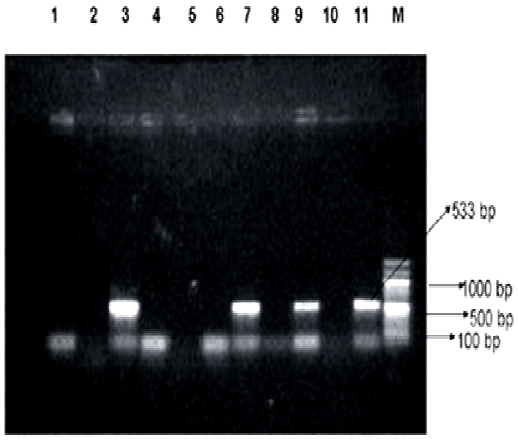

Fig. 3. MacA gene of *S. aureus* by PCR analysis

4. CONCLUSION

Staphylococcus aureus is a worldwide leading causative agent of surgical site infections (SSI). Due to the increased morbidity and mortality associated with the drug-resistant organisms, early detection and intervention are a prerequisite in surgical patients. From our pilot study with 25 samples, 9 (36%) were confirmed to be *Staphylococcus aureus* out of which 2 (22%) were found to be MRSA. *S. aureus* can cause population and health-care intrusive infections and has a wide variety of clinical syndromes, ranging from somewhat mild infections (e.g. folliculitis) to potentially life-threatening infections (e.g. bloodstream infections). It is necessary to prevent post operational infections.

CONSENT

As per international standard or university standard, respondents' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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