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Abstract

Staphylococcus species are a pathogenic group of microorganisms causing severe infectious diseases in humans and animals. Amongst the genus *Staphylococcus sp.*, *Staphylococcus aureus* is a versatile pathogen and foremost nosocomial infection causing agent. It can cause a range of illnesses, from minor skin infections, such as pimples, boils, swelling, rashes, cellulitis, folliculitis and abscesses to life-threatening diseases such as endocarditis, meningitis, toxic shock syndrome, pneumonia, osteomyelitis, bacteremia, sepsis and others. A few strains of *S. aureus* are found to be resistant to first line antibiotics raising a critical situation in clinical management of in-hospital patients by increasing the length of stay and correlated morbidities. Clinical diagnosis is critical for appropriate disease management and epidemiological surveillance. Routine microbiological practices involve the classical culture methods, which might be time consuming which is in a process to be replaced by newer molecular diagnostic methods. Modern molecular methods use analytics based on proteomic and genomic data of the micro organism for diagnosis tools. Polymerase Chain Reaction (PCR) is the most commonly used diagnostic tool which is based generally on the amplification of conserved sequences of nucleic acids. This process of shift towards molecular diagnostics has been sped up post Covid pandemic. This paper reports various advancements in the field of molecular diagnostic options available for *S. aureus*.

Keywords:

Staphylococcus aureus, Molecular Diagnostics, *S aureus* PCR detection

1. INTRODUCTION

Staphylococci are most commonly found pathogenic bacteria present in our surroundings. There are many different staphylococcal species identified. Various species of the genus *Staphylococcus* are found in different habitats, a few are found as a common part of human microbial flora, where as some have been isolated from food and animals. *Staphylococcus* species are classified upon their property to yield free coagulase enzyme. Based on this, it splits into two categories: coagulase-positive species, which are typically pathogenic, and coagulase-negative species, which are regarded less harmful. *Staphylococcus aureus* is theamong the coagulase-positive types and found to be pathogenic to both animals and humans. In recent reports, antibiotic resistance was reported in *S. aureus* typically linked with methicillin resistance *S. aureus* (MRSA) and some strains reported vancomycin intermediate (VISA) and vancomycin-resistant (VRSA) resistance as well. Approximately 20 - 30% of the global population are persistent carriers of *S. aureus*, a bacterium commonly present in the normal skin microbiota, nasal passages, and the lower reproductive tract of women [1-4]. It is among the top five most frequent causes of hospital-acquired infections and is frequently responsible for wound infections after surgery. A study unveiled that over 22% of the *S. aureus* genome is comprised of non-coding regions, which means that these regions can vary among different bacteria. An illustration of this distinction can be observed in the level of virulence exhibited by different species [5].

2. VIRULENCE FACTORS OF *STAPHYLOCOCCUS AUREUS*

Staphylococcus aureus is a notable human pathogen that induces purulent diseases, foodborne illness, pneumonia, and toxic shock syndrome, among other conditions. In addition, *S. aureus*, namely methicillin-resistant *S. aureus* (MRSA), commonly poses substantial challenges in hospitals due to nosocomial infection [6-8]. *S. aureus* produces a variety of virulence factors, including hemolysins, leukocidins, proteases, enterotoxins, exfoliative toxins, and immune-modulatory factors [9,10]. Throughout the process of development, the regulation of these elements' expression is meticulously managed. The agr system, sometimes referred to as the quorum-sensing system, is acknowledged to have a crucial function in regulating virulence factors [11]. AgrAC is a two-component system (TCS) consisting of a histidine kinase and a response regulator. The Agr system regulates the expression of various virulence factors, such as hemolysins, leukocidins, and protein A. It does so by controlling the expression of a specific gene called RNA III, which is located in the agr operon and has a divergent orientation. RNA III, in turn, regulates the expression of

multiple virulence factors [12,13]. *Staphylococcus aureus* is responsible for a diverse range of infections as a result of its virulent traits, which enable it to adhere to surfaces, infiltrate or elude the immune system, and cause significant toxic harm to the host [14].

2.1. Adherence Factors (Adhesins)

Multiple adhesins participate in the binding of S. aureus to the surface of the host cell, thereby initiating the colonization process. The microbial surface component recognizing adhesive matrix molecules (MSCRAMMs) are a group of adhesins found in *S. aureus*. They are attached to cell peptidoglycans through a threonine residue in the sorting signal motif at their C-terminus. These adhesins specifically bind to plasma or extracellular matrix (ECM) components. These molecules specifically recognize fibrinogen, fibronectin, and collagens, which are crucial constituents of the extracellular matrix (ECM) or blood plasma. Staphylococcal protein A (SpA), fibronectin-binding proteins A and B (FnbpA and FnbpB), collagen-binding protein, and clumping factor (Clf) A and B proteins all belong to the MSCRAMM family [7,15].

2.2. *S. aureus* exo-proteins

The majority of *S. aureus* strains release a range of exo-proteins, such as nucleases, proteases, lipases, hyaluronidase, and collagenase, among exotoxins and enzymes. The major function of these proteins may be to metabolize local host tissue into essential nutrients for bacterial growth [16].

2.3. Regulation of Virulence Factors in *S. aureus*

The pathogenicity of *S. aureus* is a complex process that involves the coordinated production of several extracellular and cell wall components at different stages of infection, including colonisation, evasion of host defences, growth and cell division, and bacterial spread [17]. The simultaneous expression of different virulence factors in response to environmental signals during infections such as the early expression of adhesins during colonisation and the later production of toxins to aid in tissue spread, indicates the existence of global regulators. These regulators are single determinants that control the expression of multiple unrelated target genes. These regulators facilitate bacterial adaptation to a harsh environment by producing chemicals that enable the survival of bacteria, hence promoting infection at the opportune time. Fluctuations in food availability, temperature, pH, osmolarity, and oxygen tension have the greatest capacity to modify the manifestation of virulence factors among environmental cues [17,18].

3. METHODS FOR IDENTIFICATION OF *S. AUREUS* SPECIES

Conventional methods, include selective media culture characteristics, gram staining and biochemical tests for identification of *S. aureus*. It is gram positive cocci in clusters and on visual presentation shows shiny black colonies with white margins with a surrounding clear zone on Baird-parker agar. The conventional methods for identifying and confirming *S. aureus* are time consuming. For identification and characterization, molecular methods such as PCR can be utilized for rapid identification. The PCR technique based on the 16S rRNA gene is a quick and accurate way to identify and confirm *S. aureus*. Furthermore, protein banding patterns can also be used for strain differentiation [19].

3.1. Classic Method

The classic definitive test for identification of *S. aureus* is coagulase test, majority of the strains are found positive. *S. aureus* is also frequently identified using a variety of tests, including hemolysins, thermostable deoxyribonuclease, and clumping factor. Isolation can be done by different microbial media such as Congo red modified agar, heart infusion agar, mannitol salt agar and blood agar. Other microbial flora can be inhibited by sample plating on mannitol salt agar due high sodium chloride concentration (7.5%). Another useful test for S. aureus is the synthesis of thermally stable deoxyribonuclease, which is detected by colony agglutination using coated latex particles. This coating has immunoglobulin G which binds protein A and similarly fibrinogen which has the potential to attach to the clumping factor on the surface of the bacterial cell [20].

3.2. Novel methods

Biotyping, phage typing, antibiotic susceptibility testing (AST), and multilocus enzyme electrophoresis (MLEE) are new techniques for identifying Staphylococcal strains. MLEE is not a commonly used technique since DNA-based methods are now more readily available which rely on genetic variation in housekeeping genes. Also, phage typing can be counted as less sensitive attributed to sensitivity to the phages used. Though, AST has been defined as low discriminatory power, but it has a potential to be used as an epidemiological marker in the detection of distinct antibiotic resistance amongst the isolated strains [19,20].

4. DNA BASED NON-AMPLIFICATION DETECTION METHODS

4.1. PFGE

Pulse-Field Gel Electrophoresis (PFGE) has an unique ability of enzymatic digestion of DNA molecules from the isolated microbial strains. Fingerprinting patterns of genomic DNA can be classified by PFGE which is known to be the best method to scrutinize infections. PFGE can be used to identify and compare different Methicillin-resistant *Staphylococcus aureus* (MRSA) [20,21], and is perhaps an efficient means when compared to ribotyping which does not segregate isolates into diverse subgroups unlike PFGE. Single region of chromosome is detected by ribotyping whereas PFGE detects the entire chromosome with identification of restriction sites, which makes it better than ribotyping. PFGE can be considered preeminent for *S. aureus* genotyping in terms of availability, cost, data interpretation and reproducibility [22,23].

4.2. Selective Restriction Fragment Hybridization (SRFH)

This method employs principle of southern blot hybridization with the genomic DNA. Restriction enzymes such as *Cla*I cleave the DNA molecule at specific restriction sites and assists in DNA digestion followed by agarose gel electrophoresis for the segregation of the DNA fragments [24]. This is further aided by the use of radioactive/ non-radioactive labelled probes for DNA hybridization prior to transfer on to the nylon membrane and can be used to objectify different genes. Ribotyping and prophage profiling are modified versions of SRFH for molecular typing and isolation [25-27].

4.3. Ribotyping and Ribotype Pattern Analysis

S. aureus isolates can also be segregated by the method called ribotyping in which ribosomal RNA (rRNA) gene restriction patterns are used. For an isolated strain, a restriction enzyme is used to cleave/ digest the DNA into smaller fragments followed by gel electrophoresis for segregation of rRNA genes containing DNA fragments preceding hybridization [28]. Post southern blotting, rRNA genes containing DNA fragments are identified and the developed pattern which is also known as ‘Riboprint pattern’ is observed and compared with standard values. Binding patterns of individual strain is identified and printed with the help of a device- Riboprinter. This process is crucial for subtyping of the microbial strains and monitoring the occurrence of disease-causing *S. epidermidis* strains in hospitals [29]. Ribotyping can possibly complement the phage-typing systems in *S. aureus*, but it cannot replace the traditional methods for isolating genotypes such as PFGE [30,31].

4.4. Automated Ribotyping

Various traditional methods like PFGE, plasmid pattern analysis, and polymerized chain reaction-restriction fragment length polymorphism (PCR-RFLP) which were used for identification of *S. aureus* strains, had some disadvantages such as absence of evenness, increased time and energy consumption with an added need of an exceptionally proficient workforce to handle the procedures [22-24]. Automated ribotyping, was introduced as an option which facilitates the whole process of ribotyping, overcoming these setbacks. Restriction enzyme digested DNA fragments are hybridized to form sulfonated DNA followed by detecting and capturing genetic fingerprint by the Chemioluminescent system. The images generated are analyzed by imaging analysis software and generates a ribotyping pattern. These patterns are based on the location and magnitude of the DNA fragments. Using automated ribotyping, gram-positive cocci, including staphylococci, can be quickly identified or studied for their diversity. Automated ribotyping is useful for epidemiological studies because it is typeable, easy to use, reliable and inexpensive making it an ideal methodology for microbial identification and research purposes [30,31].

4.5. Plasmid profiling

An integral part of staphylococcal genome is represented by plasmids. In *S. aureus*, the transmission of resistance to antimicrobial carriers is mainly due to accumulation of plasmids [24]. Plasmid profiling primarily focuses on alterations of plasmid content for strain identification by gel electrophoresis for molecular typing of *S. aureus* strains. Study of chromosomal DNA shows ancient relationship between the strains on the contrary plasmid content based analyses such as plasmid profiling can provide a better insight on current DNA composition and alterations making it a preferred approach which can be rapid and sensitive in *S. aureus* strain differentiation [32-34].

5. DNA BASED AMPLIFICATION DETECTION METHODS

Polymerase chain reaction (PCR) is based on target DNA content amplification, which is reported to be cost and time efficient and accurate both in terms of specificity and sensitivity. Identification of the *S.aureus* using PCR amplification of the genomic DNA is reported as one of the most effective methods for molecular identification. Different genes can be utilized for microbial molecular identification. In *S. aureus*, '*nuc'* gene is utilized majorly for the molecular detection using PCR. A '*nuc*' gene sequence is responsible for the production of the thermonuclease enzyme, which has the potential to breakdown DNA molecules. This gene can be utilized for the molecular detection and differentiation of *S. aureus* from other

staphylococcal strains which in turn can help in lining the treatment and management of *S. aureus* infections. The amplified DNA products can be used for a direct diagnosis and/ or southern blot analysis [35,36].

5.1. Gene specific PCR

Gene specific PCR can be utilized for molecular detection and identification of *S. aureus* as mentioned earlier. Apart from '*nuc'* gene other genes can also be elected and optimized for molecular detection. A research reported the detection of a product generated by using 33-mer internal '*nuc*' gene hybridization probe, which showed a positive result when the in-vitro cultured *S. aureus* was stimulated by infected *S. aureus* clinical samples were introduced and negative results were observed both in cases of samples with other bacterial infections and sterile body fluids were introduced [9,18,24,35].

5.2. Ribosomal spacer

The ribosomal spacer-PCR is a more determining and the most suitable molecular method for identification of *S. aureus* which allows a more yield and cheaper, therefore appropriate to use for regular purposes. RS-PCR simplifies the analysis as it does not involve DNA sequencing that requires high throughput RS- PCR database [42-44].

5.3. PCR RFLP

The PCR RFLP method was designed for the accurate and rapid identification of *Staphylococci*. [45] Due to various drawbacks this method is only used for species discrimination and various taxa are difficult to distinguish and leads to misidentification by phenotypic tests. To resolve this, restriction fragment length polymorphism (RFLP) and various sequencing-based methods are reported for the identification *staphylococci* [46].

In this the DNA isolation needs to be done at first which consists of centrifugation the bacterial pellet which will suspend in the lysis buffer the mixture should then be incubated for an hour maintaining the temperature 37°C.The sample should be then incubated for 10 minutes in 95°C. After centrifugation is done the DNA present in the supernant was used for amplification. Later, the 16s rRNA gene analysis is done using isolated chromosomal DNA for the identification 16s rRNA and sequence are evaluated with the data from genbank [47].

5.4. Multiple locus variable number of tandem repeat analysis

Multiple locus VNTR analysis is done for the genetic analysis of a particular organism. MLVA is also used to identify unrelated strains having duplicate pulse filled gel electrophoresis PFGE patterns[46, 47]. The genes used for *S.aureus*using this method for identification are *sdr*, *cdfA*, *cdfB*, *ssp*, *spa*. Molecular typing of MRSA strains using MLVA has also showed similarity to PFGE [48].

5.5. Arbitrarily primed PCR (AP-PCR/RAPD)

Arbitrary PCRs are proper or can be called adequate for rapid comparative typing and are not so suitable for library typing. This method uses a short primer which has random sequence at low stringency to amplification condition. Instead of using gel analysis software the PCR patterns are then evaluated visually. [49-52]

5.6. Amplified fragment length polymorphism and Infrequent restriction site amplification (AFLP and IRS-PCR)

AFLP is a selective type of amplification process of the subsets of the digested DNA. It is anovel method for DNA finger printing. Is mostly used for species and strain identification of *staphylococci*. This process involves three steps.

1) Restriction of the genomic DNA fragments and joining the adapter DNA to the fragments.
2) Now the fragments are being selectively amplified.
3) Analysis of the amplified fragments is done.

Amplification is achieved using the adapter and the restriction site as target for the annealing of the primer. Using this method, the restriction fragment can be visualized by the PCR without knowing about the sequence. [53-54]

6. CONCLUSION

S. aureus is one of the common sources of wound infections after surgery and hospital-acquired illnesses. Attributed to its virulence traits, enabling it to adhere to surfaces, elude the immune system, and induce grave toxic consequences on the host, timely diagnosis is crucial. Molecular detection of *S. aureus* can greatly facilitate the medical intervention needed on the basis of its conservative genetic traits.

7. ACKNOWLEDGEMENT :- Not Applicable

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AUTHOR STATEMENT

MG- Manuscript first draft, data analysis; HG- Manuscript first draft, data analysis; PK- Manuscript first draft, Data analysis; PP- Conceptualization, Resources, Manuscript review; SKM- Conceptualization, Manuscript review and editing; SBn-Manuscript first draft, methodology; SBa-Manuscript first draft, methodology, Formatting.

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