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Isolation and Biochemical Charecterization of Staphylococcus Aureus Isolated From Davangere District

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Abstract

Staphylococcus aureus has persisted and is now resurging as an important hospital and community acquired pathogen. The pathogen could result the wide spectra of pathogenic diseases that could include bacteremia, endocarditis, osteomyelitis, and the nosocomial infection. Although a remarkable progress has been made in the arena of development of antibiotics, still the community acquired and nosocomial infections remain significant and formidable consequence of hospitalization. Epidemiologically important *Staphylococcus aureus* was isolated form different clinical samples. An effort was made to collect the sample from five different places of davangere region, for convenience, places were classified into five Zones. In this study we have isolated 150 samples and out of which 60 samples were confirmed by biochemical and distinguishing tests as positive samples of *Staphylococcus Aureus*.

Introduction

Staphylococcus aureus can cause infections commonly in newborns, surgical, burns, diabetic patients, and persons who are taking drugs suppressing the immunodeficiency diseases. Although *S. aureus* is an important pathogen, many healthy people may carry it as a part of the normal microflora associated with the nose, throat, perineum or skin. The individuals who are asymptomatic nasal carriage of *S. aureus* are at risk of developing persistent nasal carriage and thus they can disperse the organisms into the environment around them. Many studies showed that there is a dramatic increase of *S. aureus* that are resistant to multiple antibiotics which poses an increasingly serious problem in many hospitals, and it is responsible for numerous hospital outbreaks.

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There are many conventional techniques used for isolating and identifying this bacterium, such as biotyping, antibiotic typing and phage typing. Recently strains of multiple drug resistant *S. aureus* have appeared and proven very difficult to treat. During the late 1950s and early 1960s, *Staphylococcus aureus* caused considerable morbidity and mortality as a nosocomial or hospital acquired pathogen and has become the leading cause of nosocomial infection during the last 2 decades. Since then penicillinase resistant semi synthetic penicillin as proved to be successful antimicrobial agents in the treatment of *staphylococcus* infections. Unfortunately, MRSA (methicillin resistant *Staphylococcus aureus*) strains isolated are on increasing resistant to multiple non-β-lactam containing antimicrobial drugs. Isolation and biochemical studies is an important to understand the spread of the disease.

Material and Methods: Sample Collection Zones:

Isolation and study of epidemiology of *Staphylococcus aureus* from the infected patients were chosen from five places of Davangere district, Karnataka. The Davangere district temperature varies between 25 to 39° C. The places

were classified in to five Zones as A (District Govt. Hospital, Davangere), B (Channagiri Govt. Hospital), C (Diagnostic Laboratory Davangere) D (Govt. Hospital Honnali) and E (Govt. Hospital Jagaluru). Clinical samples were collected regularly from above mentioned places during January 2018 to December 2019.

Isolation of *Staphylococcus aureus* from different clinical samples:

Collection of Samples:

The clinical samples were collected form the patients who were infected with *Staphylococcus aureus* in and around Davangere region of Karnataka state, India. The clinical specimens like pus, cerebrospinal fluid (CSF), blood, urine, biomedical waste were selected as the sources of organisms and carried in 18.2% peptone water to the laboratory. Mannitol salt agar was used as a selective media for primary isolation of the *Staphylococci.*

Media used for isolation and Characterization of *Staphylococcus aureus*:

For the present study different culture media like, Nutrient agar, Mannitol salt agar (MSA), Brain heart infusion agar (BHI) and Blood agar were used. Nutrient agar/broth was prepared for the appropriate growth of *Staphylococcus aureus* culture, which could be the source of the strains for inoculation in subsequent steps. MSA plates were prepared and streaked; the inoculations could be done either from the peptone water containing organisms or from the nutrient broth culture.

Colony Characterization and Microscopic Observation:

The isolated colony from mannitol salt agar (MSA) was picked up and prepared the smear on grease free glass slides, which was dried and heat fixed. Gram-staining was performed according Christiana Gram which allows better differentiation of organisms. The microscopic observation showing spherical cocci, 1-1.5 μ m in diameter occurring singly, in pairs, short chains >7 cocci, or in small groups resembling a cluster of grapes were considered as *Staphylococci.*

Biochemical Characterizations of *Staphylococcus aureus*:

Preparation of Inoculums:

Organisms were isolated on a nutrient agar medium. Single isolated colony was taken and inoculated in 50 ml of brain heart infusion broth, incubated for 6 hours at 37°C such that inoculums turbidity was greater than 0.1 O.D. at 620 nm or 0.5 McFarland standards. Kit was opened aseptically where by seal were removed. Each test well was inoculated with 30 µl of the inoculum by surface inoculation method. The test strips were incubated at 37°C for 18-24 hrs.

Voges-proskaeur's test:

Some organisms have the ability to produce a neutral end product acetyl methyl carbinol (acetoin) from glucose utilization. This can be detected by addition of 1-2 drops of Barrit Reagent (R029) and 1-2 drops of Barrit Reagent B (R030). A positive test is indicated by pinkish red color within 2-5 min. No change in color indicates negative test.

Alkaline phosphatase test:

This test detects the ability of microorganism to produce sufficient phosphatase enzyme. Phosphatase production is determined by liberation of phenolphthalein. The liberated phenolphthalein reacts with alkali (40% NaOH) to give a bright pink color.

ONPG test:

Two enzymes, Permease and galactosidase are required for Lactose fermentation. True non-lactose fermenters are devoid of both enzymes, however some organisms may lack Permease but posses the enzyme β-galactosidase. ONPG (o-nitrophenyl-β-D-galactopyranoside) is structurally similar to lactose. In the presence of galactosidase, ONPG is cleaved into galactose and o-nitrophenol, a yellow compound. Since members of are routinely grouped according to their abilities to ferment lactose, the ONPG test is especially useful in rapidly identifying cryptic lactose fermentation. Development of a yellow color when ONPG Discs (DD008) is placed on 8-24 hrs growth and incubated further for a minimum of 1 hr at 35^0C indicates positive reaction.

Urease test:

This test detects the ability of an organism to split urea to ammonia by the action of enzyme urease. In case of positive test, the medium turns pink under alkaline conditions due to phenol red indicator in the medium. No change in color indicates negative reaction.

Arginine utilization test:

The medium for this test contains Bromocresol purple as pH indicator. When carbohydrate present in the medium is utilized, pH is lowered due to acid production changing the color of medium to yellow. The acid produced stimulates decarboxylase enzyme. The formation of amine due to this reaction increases the pH of the medium, changing the color of the indicator from olive green to purple. Negative reaction is indicated by development of yellow color.

Carbohydrate utilization test:

Mannitol, Sucrose, Lactose, Arabinose, Raffinose, Trehalose and Maltose all these specific carbohydrates are added to basal media which contains phenol red as indicator. On fermentation of carbohydrate, acid is liberated which lowers down the pH of medium and this change of color is indicated by pH indicator dye. Positive test is indicated by color changes from red to yellow due to acid reaction.

Distinguishing characterization of *Staphylococcus aureus:*

Blood hemolysis test:

The pathogenic organisms are able to lyse the red blood cells (RBCs), 10 ml of non coagulated sheep blood with 10mM EDTA (anti-coagulating agent) was added to sterilized nutrient agar medium plates were streaked with isolates and incubated at 37°C for 24 hrs. A Zone of clearing was observed around colonies, which indicates hemolysis of RBCs.

Catalase test:

The catalase test was done by transferring few drops of 18 hrs. nutrient broth culture on to a slide with 3% hydrogen peroxide (H_2O_2), which shows immediate vigorous gas

bubbles from the bacterial culture is due to the liberation of O_2 from H_2O_2 by the action of the catalase enzyme.

Coagulase test:

Coagulase test is used to distinguish *S. aureus* from other sub species of *Staphylococci.* 1 ml of human plasma is diluted with 0.85% NaCl (1-in-6) was placed in small tubes. Isolated colonies were emulsified into diluted plasma. Tubes were incubated at 37^0 C in water bath up to 4-6 hrs later tubes were examined at 1, 2, 4 and 6 hrs and observe clot formation by tilting the tube at 90^0 known coagulase-positive and coagulase-negative cultures were taken as controls, a tube of unseeded diluted plasma to confirm that it does not clot spontaneously.

DNase test:

The DNase test was helpful in identifying strains that give doubtful reactions in tube coagulase test agar plates were divided in to 4-6 sections by drawing lines on its bottom and each section were numbered to denote the applied strains. Spot–inoculation of *S. aureus* isolates was made onto a small area of medium, so as to produce a thick disc of growth. The plates were incubated aerobically at 37^0 C for 18-24 hrs and then each plate was flooded with few ml of 1 M (3.6%) hydrochloric acid to precipitate unhydrolysed DNA. Plates were examined against dark background, the spot cultures surrounded by clear, Zones were considered as DNase-positive culture. Known DNase-positive and Dnase-negative cultures were used as controls.

Antimicrobial Susceptibility test:

Antibiotics susceptibility was performed as per reliability and reproducibility guidelines of Clinical Laboratory Standard Institute (CLSI), and quality assurance guidelines of World Health Organization (WHO). The method adopted was the Kirby-Bauer's Agar disk diffusion assay for antibiogram. The antibiotic susceptibility discs and the Mueller Hinton agar were obtained from Hi-Media Laboratories, Mumbai.

Results:

Isolation and Characterization of *S.aureus:*

The present investigation was carried out during the period from January 2018 to November 2019. During this period of time epidemiologically important *Staphylococcus aureus* was isolated form different clinical samples. An effort was made to collect the sample from five different places of Davangere region. For convenience, places were classified into five Zones.

A total of 150 clinical samples, from 100 males and 50 females were collected. For isolation and identification of *Staphylococcus aureus*, samples were inoculated on to the selective medium viz: Manitol Salt Agar (MSA), Brain Heart Infusion Agar (BHI) and Blood Agar. Distinguishing colonies grown on both media at 37^0C were picked and confirmed by microscopic and biochemical characterization.

Out of 150 clinical samples inoculated, 60 (55.00%) strains were confirmed as *Staphylococcus aureus* and were further characterized. The microscopic studies for all strains displayed gram positive cocci, arranged in clusters, non-motile and non-spore forming. The results of biochemical reactions of the isolates were positive for Voges Proskauer's, Alkaline phosphate, O-nitrophenyl-β-D-galactopyranoside (ONPG), and Catalase production. The isolates were also found positive for all the carbohydrates used in the HiStaph Identification kit (Kb-004). The growth on selective media indicated the fermentation of mannitol salt agar β-haemolysis of sheep blood cells on blood agar and production of DNase and coagulase confirms it as coagulase positive.

Discussion*:*

S. aureus is a facultative anaerobic gram positive bacteria appears as grape-like clusters when observed under simple microscope and has large round and golden-yellow colonies often with haemolysis were observed when grown on blood agar plates. *S. aureus* is catalase-positive which is able to convert hydrogen peroxide (H_2O_2) to water and oxygen, which makes the catalase test useful to distinguish staphylococci from enterococci and streptococci A small percentage of *S. aureus* can be differentiated from most other staphylococci by the coagulase test, *S. aureus* is primarily coagulase-positive because it can produce the enzyme coagulase that causes clot formation

Summary and Conclusion:

The *S. aureus* is a major problem in medical care, due to increased drug resistance to almost all the traditional antibiotics, including class of β-lactams (Methicillin antibiotic). The growing threat from resistance strains calls for development of accurate diagnostic methods and effective treatment strategies. Recent studies have focused on molecular mechanisms of antibiotic resistance to understand spread of antibiotic resistance genes among the species. To treat *S. aureus* infections in convincing way there is a need of novel strategies/approaches to find better drug candidate in the field of pharmaceuticals. In this study we made an effort to collect the samples from five different zones of Davangere district, isolated in laboratory and understand the spread of the organisms at different zones of the district and we confirm the organism by different biochemical analysis out of 150 samples 60 sample are found to be *S. Aureus* samples.

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