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Pathogenic *Staphylococcus aureus* Isolates from Postoperative Wounds of Hospitalized Patients.

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Abstract: *Staphylococcus sp*., gram positive pyogenic bacteria located on skin, nose etc, secretes toxin that causes toxic shock syndrome, abscess, food poisoning and other infectious diseases. This study was carried out to identify and characterize the type of *Staphylococcus sp*. bacteria especially *Staphylococcus aureus* in the pus from postoperative wounds of hospitalized patients. From pus samples collected from twenty-four patients from Kharagpur Hospital, Paschim Medinipur, West Bengal, twenty-eight bacterial isolates were obtained. Among them twenty-five (89.2%) were appeared with golden yellow colonies which is usually formed by *Staphylococcus aureus*. Twenty-three (82.14%) of the bacterial isolates were Gram positive. Among them twenty isolates (86.9%) were further confirmed to be *Staphylococcus aureus* by their ability to produce Catalase enzyme (positive in Catalase test) and Coagulase enzyme (positive in Coagulase Test). Eighteen (90.00%) of these *Staphylococcus aureus* were found to liquefy gelatin (Gelatin hydrolysis test), were able to hydrolyze urea (Urea hydrolysis test) and were also l positive in Mannitol Fermentation Test. But there was no growth found of these isolates on MacConkey Agar, while sixteen isolates (80.00%) of *Staphylococcus aureus* were resistant to penicillin (50µg/ml). Moreover eighteen (90.00%) Staphylococcus aureus isolates were able to elaborate Hemolysin (Hemolysis test on Blood Agar media). Hence the bacterial isolates obtained from pus of postoperative wounds were predominantly pathogenic Staphylococcus aureus. So it can be concluded that careful treatment and postoperative measures to be taken to avoid serious health problem that may often be life threatening.

Key Words: Pus, Post Operative Wounds, *Staphylococcus aureus*, Catalase, Coagulase Hemolysin

Introduction

Staphylococcus aureus is a ubiquitous bacterium that is generating increasingly bad press coverage due to its propensity to adopt a pathogenic lifestyle in hospital and community settings. It colonizes the skin readily and can lead to a wide range of pathological conditions from, skin lesions to osteomyelitis, endocarditis, and septicemia. Drug resistance among *Staphylococcus* isolates amplifies this escalating problem. One of the pathological consequences of *Staphylococcus aureus* infection is the development of skin abscesses characterized by the extensive recruitment of neutrophils. Neutrophils are the front-guard in response to bacterial invasion and are equipped with an arsenal of antimicrobial agents [1]. *Staphylococcus aureus* causes pyogenic infection in man and is the common cause of boils, carbuncles, impetigo and infection of surgical or accidental wounds and burns [2]. Various forms of trauma resulting around superficial abscess such as squeezing a boil may force large number of *Staphylococcus* into the blood from where they are carried into many tissues of the body, thus causing systemic infections and abscesses may be produced

in various deep organs such as liver, lung or brain tissues. *Staphylococcus aureus* may settle in the lungs to cause pneumonia or in pelvis of the kidney to cause polynephritis] [3-4]. *Staphylococcus aureus* also causes toxic shock syndrome and scalded skin which is usually started with high fever, vomiting and diarrhea. In some cases kidney failure and a red skin rash may be noted. Without any prompt supportive treatment death may be caused. Most cases in females are observed during or immediately after menstrual period [5-6]. Food poisoning is an intoxication results from the ingestion of food containing preformed *Staphylococcal* enterotoxin [7]. *Staphylococcus aureus* elaborates an enzyme called coagulase which activates clotting mechanism, *Staphylococcus aureus* that does not secrete coagulase are of low or no virulence [8]. An attempt has been made in the present study to identify and characterize pathogenic *Staphylococcus aureus* organisms from wounds of hospitalized patients those may be life threatening.

Materials and Methods

Collection of sample: Pus samples were collected from the postoperative wounds of twenty-four adult male patients from the hospitals of Kharagpur (Paschim Medinipur District, West Bengal) with informed consent of the patients and the hospital authorities. Pus samples were collected aseptically and transferred into appropriate media and incubated overnight at 37ºC. The bacterial colonies obtained from the pus samples were undergone a number of following tests for their characterization. *Screening through Gram staining:* Bacterial isolates obtained from the pus were screened by using Gram stain [9]. Gram positive strains appeared in violet colour and Gram negative bacteria appeared in red colour.

Biochemical characterization : *Urea Hydrolysis Test:* The test for Urea hydrolysis test was performed by the standard method [9]. Bacterial cells were grown on media specified for urea hydrolysis test (2 gm% urea, 1.5gm% agar, 0.5 gm% NaCl, 0.2% K_2HPO_4, 0.0012 gm% phenol red). Bacterial culture appeared in pink coloration are taken to be positive for hydrolyzing urea. *Gelatin hydrolysis Test:* Gelatin hydrolyzing ability of bacterial isolates was screened through gelatin hydrolysis test by the method of gelatin hydrolysis test [9]. Bacterial cells were allowed to grow on the specific media for gelatin hydrolysis test (0.3% beef extract, 0.5% peptone, 12% gelatin). Gelatin liquifying bacterial isolates are taken to be positive for hydrolyzing gelatin. *Lactose Fermentation & Penicillin Resistance:* Bacterial isolates were screened by MacConkey agar media [9] and agar media containing penicillin (50µg/ml) separately. Bacterial colonies appeared in red to pink coloration were taken to be positive in MacConkey agar test for lactose fermentation. Cultures growing on agar with penicillin are considered to be penicillin resistant. *Mannitol Fermentation:* Mannitol hydrolyzing ability of the isolated bacterial cells were determined by the standard method [9]. Bacterial cells were grown on the media specified for Mannitol test (7gm% NaCl, 2.3 gm% Nutrient agar, 2 gm% Luria Broth, 1gm% Mannitol and 0.0025 gm% phenol red). Cultures appeared as yellow colonies were taken to be positive for Mannitol fermenting test. *Catalase Activity:* To identify catalase activity in the isolated bacteria Catalase test was performed [7].

Bacterial cultures having the ability to produce gaseous bubbles after addition of H_2O_2 were taken to be positive for secreting ability of catalase enzyme.
Tests for Pathogenesis: Coagulase Activity: Coagulase expression ability was studied by the standard method for Coagulase test [9]. Presence of visible clumping of cells after emulsification of bacterial cells with plasma indicated that cultures were positive in coagulase secretion. *Hemolysis:* To identify the hemolytic ability of the bacterial isolates were screened on Blood agar media by the method of hemolysis test [9]. Bacteria having the hemolytic ability produce a zone of clearing surrounding the colony (α haemolysis), β hemolytic bacteria produces partial (green clearing) zone surrounding the colony and bacterial isolates which have no hemolytic ability surrounding the colony are γ hemolytic [10], Clearing zone producing bacterial isolates on Blood agar media were taken to be positive for hemolysing blood.

Results

Twenty eight bacterial isolates were identified from pus samples of twenty four hospitalized patients. Among them twenty five (89.2%) [Table 1] isolates were found to form smooth golden yellow colonies [Fig-1] which may be *Staphylococcus aureus*. In gram staining twenty three (82.14%) isolates were identified as gram positive [Table 1] and twenty (86.95%) isolates of these gram positive yellow colonies were positive for catalase and coagulase activities [Table 2]. Hence these twenty bacterial isolates with golden yellow colonies those were also positive for catalase and coagulase activities were confirmed to be *Staphylococcus aureus*. Eighteen (90.00%) of these *Staphylococcus aureus* were also able to hydrolyze urea and gelatin [Table 3]. These *Staphylococcus aureus* were not able to grow on MacConkey agar. Sixteen (80.00%) of them were able to grow on penicillin (50µg/ml) containing agar [Table 3]. Eighteen (90.00%) *Staphylococcus aureus* isolates were found to be hemolytic on blood agar media and were also Mannitol fermenting [Table 3].

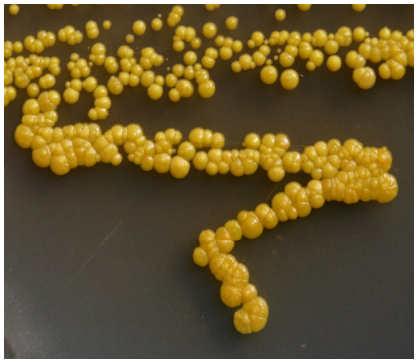

Figure -1: Golden yellow colonies of isolated *Staphylococcus aureus* from pus sample from postoperative wound of a patient

Table 1: Bacterial Screening through Colony Morphology and Gram Stain

Bacterial isolates	Yellow colonies	Percent of bacteria with yellow colonies (%)	Gram staining	Percent Gram Positive (%)
S1	+	89.2	+	82.14
S2	+		-	
S3	+		+	
S4	+		+	
S5	-		-	
S6	+		+	
S7	-		-	
S8	+		+	
S9	+		+	
S10	+		+	
S11	+		+	
S12	+		+	
S13	+		-	
S14	+		+	
S15	+		+	
S16	+		+	
S17	+		+	
S18	+		+	
S19	+		+	
S20	+		+	
S21	+		+	
S22	+		+	
S23	+		+	
S24	+		+	
S25	+		+	
S26	+		+	
S27	+		+	
S28	-		-	

Table-2: Tests for identification of pathogenic *Staphylococcus aureus*

Yellow gram positive isolates	Catalase Activity	Percent positive for catalase activity (%)	Coagulase Activity	Percent positive for coagulase activity (%)
S1	+	86.95	+	86.95
S3	+		+	
S4	-		-	
S6	+		+	
S8	+		+	
S9	+		+	
S10	+		+	
S11	+		+	
S12	+		+	
S14	+		+	
S15	+		+	
S16	+		+	
S17	-		-	
S18	+		+	
S19	+		+	
S20	+		+	
S21	+		+	
S22	+		+	
S23	+		+	
S24	+		+	
S25	+		+	
S26	+		+	
S27	-		-	

Table-3: Biochemical tests for characterization and pathogenecity detemination

Isolates of *Staphylococcus aureus*	Urea hydrolysis test	Gelatin hydrolysis test	Growth on MacConkey agar	Growth in Agar with Penicillin (50µg/ml)	Mannitol Fermentation Test	Hemolysis Test
S1	+	+	-	+	+	+
S3	+	+	-	+	+	+
S6	+	+	-	+	-	+
S8	+	+	-	-	+	+
S9	+	+	-	+	+	+
S10	+	+	-	+	+	+
S11	+	+	-	+	+	+
S12	+	+	-	+	+	+
S14	+	+	-	+	+	+
S15	+	+	-	+	+	+
S16	+	+	-	-	+	+
S18	-	-	-	+	+	-
S19	+	+	-	+	-	+
S20	+	+	-	+	+	+
S21	+	+	-	-	+	-
S22	+	+	-	+	+	+
S23	+	+	-	+	+	+
S24	+	+	-	+	+	+
S25	+	+	-	+	+	+
S26	-	-	-	-	+	-
Percent (%) positive	90.00	90.00	00.00	80.00	90.00	90.00

Discussion

Staphylococcus aureus is a common pathogen responsible for nosocomial and community infection. It readily colonizes indwelling catheters forming microbiotic communities termed biofilms in which *Staphylococcus aureus* are protected from killing by antibiotics and body's immune system. For years, one mechanism behind biofilm resistance to attack from the immune system's sentinel leukocytes has been conceptualized as a deficiency in the ability of the leukocytes to penetrate the biofilm [11]. As an opportunistic pathogen, it can cause infections that vary widely in their susceptibility to antibiotic treatment. The variability is caused by differences in the gene content among the strains. *Staphylococcus aureus* is resistant to vancomycin [12] and also to methicillin [13]. In this present study pus samples were collected from the post operative wounds of twenty-four hospitalized patients from Kharagpur, Paschim Medinipur District, West Bengal. Twenty eight bacterial isolates were identified, among them twenty five (89.2%) isolates were found to form smooth golden yellow colonies [Fig-1] which may be *Staphylococcus aureus*. *Staphylococcus aureus* produces round raised opaque colonies that usually have a golden-yellow colour [10]. The golden color is the result of the production of a lipid pigment contained in the organism. The species name *Staphylococcus aureus* (golden, in Latin) for its characteristic surface pigmentation in comparison with less virulent *staphylococci* that normally colonize the skin surface [14]. Subsequent studies of the S*taphylococcus aureus* pigment have unraveled an elaborate biosynthetic pathway that produces a series of carotenoids [15]. It was hypothesized that *Staphylococcus aureus* could utilize its golden carotenoid pigment to resist oxidant-based clearance mechanisms of the host innate immune system [16]. Then these isolates were screened with gram stain and it was observed that twenty-three (82.14%) [Table1] of these isolates appeared with violet colour under microscope and were considered as gram positive. Among twenty eight initial isolates from pus samples twenty three (82.14%) appeared to be *Staphylococcus aureus* bacteria depending upon initial screening through colony character and gram staining. For the confirmation of *Staphylococcus aureus* bacteria catalase and coagulase activities were studied. Twenty isolates (86.95%) [Table 2] with golden yellow colonies were found to produce bubbles from H_2O_2 due to the production of O_2 by the action of catalase enzyme produced by them. Beside this when single colony of each of these catalase positive isolates (86.95%) was added to rabbit plasma and emulsified, the cells were clumped. Coagulase is an enzyme which is characteristically produced by *Staphylococcus aureus*. Coagulase reacts with the prothombin in the blood and causes blood to clot by converting fibrinogen to fibrin. Coagulase is tightly bound to the surface of *Staphylococcus aureus* and coats its surface with fibrin upon contact with the blood. It has been proposed that coagulase positive *Staphylococci* making the bacteria more virulent and so this test is used to differentiate pathogenic *Staphylococcus aureus* from coagulase negative nonpathogenic *Staphylococci aureus* [8, 17-18]. So the result of coagulase test confirmed that those bacterial isolates who were positive in Coagulase test were *Staphylococcus aureus*. Then Urea hydrolysis, Gelatin hydrolysis, Lactose fermentation and Mannitol fermentation tests were performed to characterize

Staphylococcus aureus. So, these were allowed to grow on gelatin containing media (gelatin hydrolysis test) and about eighteen (90.00%) of these *Staphylococcus aureus* were found to liquefy gelatin. This was due to secretion of gelatinase enzyme from their cell wall which liquefied gelatin into soluble carbohydrate. When urea hydrolysis test was performed with these isolates they were found to form red to pink colonies. It may be due to liberation of ammonia, the end product of urea hydrolysis by the enzyme urease secreted from their cell wall [Table-3]. No growth of *Staphylococcus aureus* isolates was found on MacConkey media, because they can not hydrolyze bile salt and crystal violet. But sixteen (80.00%) *Staphylococcus aureus* isolates were found to grow on LB Agar plate containing penicillin (50μg/ml) [Table-3]. Hence these were resistant to penicillin and also nonlactose fermenting bacteria. But uease positive *Staphylococcus aureus* (90.00%) isolates were also Mannitol fermenting bacteria as they fermented Mannitol and produced yellow colonies. For determining the pathogenic ability of the coagulase positive *Staphylococcus aureus* hemolysis test using blood agar media was performed and eighteen (90.0%) of these coagulase positive bacteria produced clearing zone surrounding their growth [Table-3]. In blood agar media blood was incorporated into medium to provide growth factor required by the fastidious pathway of bacteria those have hemolysing power of blood (RBC) by secreting enzyme hemolysin. Hemolysing bacterial isolates produce a clearing zone surrounding the bacterial colony (complete hemolysis –α hemolysis, partial hemolysis- β hemolysis and low or no hemolysis – γ) [19]. Hence it is clear from this study that the post operative wounds are infected with pathogenic and harmful *Staphylococus aureus* bacteria which will definitely be life threatening problem if it is not properly taken care of.

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