Comparison of protein — A with clumping factor, hemolysis and coagulase tests for identification of Staphylococci isolated from Nasal swabs of healthy carriers

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Abstract:

Thirty nine (12.8%) isolates of Staphylococcus aureus were isolated from 304 healthy human (Nasal swabs). It was found that percentage of males that have S. aureus is more than female's percentage. These isolates (39) were tested with different tests. Twenty seven isolates (69.23 %) were positive for Staphylococcus protein —A (SPA) ,thirty seven (94.8 %) were positive for tube coagulase , thirty five (89.7 %) were positive with clumping factor and thirty two (82.05 %) had 13 — hemolytic on blood agar. It was found that 100% of the isolates (39 isolates) were positive with one, two or three tests (tube coagulase, clumping factor and SPA).

Introduction

The genus Staphylococcus contains 32 species and 15 subspecies which are widespread in nature (l).Colonies are usually opaque and may be white , creamy and sometimes yellow .The nitrate is often reduced to nitrite and usually grow with 10% NaCI .The optimum temperature is 30 - 37°C (2). Medically, only three of them are significant human pathogens, S.aureus which causes various pyogenic infections (endocarditis, osteomylitis, skin and soft tissue infection toxin mediated diseases such as food poisoning, toxic shock syndrome and the scalded skin syndrome) S. epidermidis and S. saprophyticus that cause urinary tract infections (3) .The catalase test is important in distinguishing Streptococci (negative catalase) from Staphylococci (positive catalase)(3).

Several studies have been carried out to determine the biochemical properties of Staphylococcus isolated from human. Tube coagulase ,clumping factor, protein-A ,deoxyribonuclease thermonuclease, caseinase and lesitinase are commonly used for the identification of Staphylococci (4).The test for clumping factor was found to be suitable for identification of coagulase positive Staphylococci , the test can be easily performed and more economic compared to tube coagulase test (5). Protein-A is a protein on cell wall of some S. aureus strains and several studies have been done on the protein-A activates of Staphylococci isolated from human and animals of various origins (6, 7). The common bacterial pathogen (S. aureus) produces a 42 KDa factor. Staphylococcus protein—A (SPA) can bind with Fc and Fab of immunoglobulin

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(Ig.) (8). S. aureus is an important causative agent of nosocomial infections including surgical site infections and catheter — related bacteremia (9) Between 20 — 70 % of adult individuals carry S. aureus in the nose , some of these individuals are permanently colonized and others are only transiently colonized .Nasal carriers commonly have S. aureus on other body sites as well (10,11,12). S. aureus nasal carriage has been extensively studied in patients and healthy individuals (13, 14) because S. aureus nasal carriage may play an important role in outbreak of S. aureus infections exactly in medical and non medical people (15).

The study aimed to compare among many essential tests that are used in the diagnosis of S. aureus, with the attempt to establish a good test for the diagnosis of this bacterium.

Materials and Methods:

Healthy carriers: A total of 304 adults (120 males and 185 females) were investigated, and their ages were 21-52 years .All of them were students and lecturers at The University of Baghdad.

Sampling and isolation of *S. aureus*: Samples were taken from noses by cotton swabs. The swabbed samples were directly inoculated onto Staphylococcus 110 (Selective media) and incubated at 37°C for 24 h.

Identification of *S.aureus*: Colonies on the Staphylococcus 110 were subcultured on the mannitol salt agar and blood agar and incubated at 37°C for 24 hour. The Identification of yellow colonies to *S. aureus* dependent on Bergy's Manual of Determinative Bacteriology (2) . . .

Determination of Protein —A activity: Fresh cultures of *S. aureus* were grown on Mueller Hinton agar for 18-24 h. and the colonies were suspended in 25µl of Latex reagent coated with IgG (Bio-Kit for detection of protein -A) on slides . The formation of agglutination within 2 minutes was considered as a positive result, positive and negative controls were also included.

Tube coagulase test: 0.1 ml of fresh cultures of *S. aureus* grown on Nutrient broth for 18 — 24 h. was added to 0.5 ml of 1 / 10 diluted sterile rabbit plasma in test tube. The tube was incubated at 37 °C and the presence of coagulation was observed after period of 2, 4, 6, and 24 hour. Positive and negative staphylococci were also used as controls (16).

Clumping factor test :Fresh culture (colonies) of *S. aureus* grown on Muller Hinton agar for 18-24 hours were suspended in 25µl of rabbit plasma and presence of clumping within 30 seconds or 2 minutes was considered as a positive result .Positive and negative controls were also done (17) .

Results and discussion:

Thirty nine isolates of *S. aureus* were isolated from the investigated subjects. Twenty isolates from females and 19 from males but the percentage of males that had *S. aureus* from total number of males was more than this percentage in females (Table-1-). Many reports have described the rate of nasal carriage in various populations (l0, 18). The current study showed low incidence (12.8 %) than other studies (10, 1*8)* because *S. aureus* was isolated from very educated population (University students and staff), The percentage of *S. aureus* incidence in males was found more than females because males in middle east populations is more contact together than

females in the same population, that is meaning more transmission of *S. aureus* from carriers to another. *S. aureus* nasal carriers have been established at a major risk factor for the development of both community and nosocomial infections.

Table - 1 – Observed numbers and percentage frequencies of cases that had S.aureus in their noses.

Cases with S. aureus in their noses	Values
Number of individual	304
Range of ages in years	21 -52
Number of males	120
Number of females	184
Number and percentage of cases that had *S. aureus* in their noses (total)	39 (12.8 %)
Number and percentage of males that had *S. aureus* in their noses	19 (15.8%)
Number and percentage of females that had *S. aureus* in their noses	20 (10.89 %)

The results obtained by latex agglutination for protein —A, clumping factor, tube coagulase and hemolysis on blood agar are given in Table -2- .

Table -2- Tests used for detecting 39 *S. aureus* isolates that isolated from nasal swabs

Tests	Positive tests	
	Number	Percentage
Staphylococcus protein—A	27	69.23 %
Tube coagulase	37	94.8 %
Clumping factor	*35*	89.7 %
Hemolysis on blood agar	32	82.05 %
Tube coagulase or/and SPA	38	97.43%
Clumping factor or/and SPA	37	94.8 %
Hemolysis on blood agar or/and SPA	38	97.43 %
Tube coagulase or/and clumping factor or/ and SPA.	39	100%

It was documented that 27 (69.23%) isolates were positive for Staphylococcus protein —a (SPA) , 35 (89.7 %) were positive for clumping factor 37 (94.8 %) were positive for tube coagulase , 32 (82.05 %) were positive for hemolysis on blood agar , 38 (97.43 %) were positive for tube coagulase or/and SPA , 37 (94.8%) were positive for clumping factor or/ and SPA and 38 (97.43%) were positive for hemolysis or/and SPA .But 39 (100 %) were positive for tube coagulase or/and clumping factor or/and SPA . Many studies have been carried out on the determination of the biochemical and biological characteristics of Staphylococci isolated from human of different origins (10, 11 , 19 , 20) .Some differences were found between the present results and other results that have been found by other investigators (19 , 20). It was found a total agreement between (Clumping factor or/and SPA) and tube coagulase test for identification of *S. aureus* .The same results were noted by other investigators (21).All SPA positive isolates were positive with tube coagulase or clumping factor . The same results were found by other investigators in human but these results were different in animal (22) .The isolates of S. *aureus* which give a positive test in B-hemolytic on blood agar or/and SPA had the same the results in tube coagulase or/and SPA. This study documented that all isolates of *S. aureus* isolated from nasal swabs gave positive results with Clumping factor or/and tube coagulase or/and SPA. Medical laboratories world wide play a key role in patient's management as physicians demand and depends upon laboratory tests and investigation to support their diagnosis and as a basis for the treatment of their patients (3).Not all isolates gave positive results with coagulase test (tube

test) because number of strains produces fibrinolysin which lyses the clot within the first four hours (23) so these strains would have been misdiagnosis as coagulase negative Staphylococci , if the clot were not observed .Not all strains of S. aureus produces bound coagulase ,hemolysin and have SPA on their cell wall (about 95 % of *S aureus* strains and this protein has an ability to bind to the Fc region of immunoglobulin (IgG)).(3, 24), so there is no one test gave positive result with all strains of *S. aureus* so if we want to reach a good way to diagnose this bacterium , many test must be used together to reach our aim. The basic bacteriological principles must be observed such as colony morphology, Gram stain reaction and pure culture technique before performing any biochemical tests these will certainly help to minimize error, for instance; some virulence strains of *Yersinia pestis* are coagulase positive (25).

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مقارنة بروتين –أ مع عامل التكتل و التحلل الدموي واختبار التجلط النبوبي لتشخيص العزلأت العنقوية المعزولة من مسحات انوف اشخاص اصحاء

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الخلأصـة:

عزلت تسعة وثلأثون عزلة (12.8 %) من العنقوديات الذهبية من انوف ثلأثمائة واربعة اشخاص اصحاء . وجد ان نسبة الرجال الحاملين لهذه العزلأت بالنسبة الى اعدادها كانت اكثر من نفس النسبة لدى النساء .اختبرت جميع العزلأت بعدة اختبارات حيث وجد ان 27 عزلة (69.23%) تعطي نتيجة ايجابية مع اختبار الكشف عن بروتين –أ و37 عزلة (94.8%) اعطت نتيجة يجابية مع اختبار التجلط الأنبوبي و 35 عزلة (89.7%) اعطت نتيجة ايجابية مع اختبار عامل التكتل و 32 عزلة (82.05%) حللت الدم (تحليل تام) . من هذه الدراسة وجد ان 100% (39 عزلة) اعطت نتيجة ايجابية مع واحد او اثنين او ثلاث من الاختبارات الاتية (فحص البروتين – أ و عامل التكتل و اختبار تجلط الأنبوبي).