

Study Some Virulence Factors of CO-agulase Positive Staphylococci and CO-agulase Negative Staphylococci Isolated from Different Parts of Human Body

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Abstract:

This study included test production of some virulence factors that product from some species of Staphylococci: Co-agulase Positive Staphylococci (COPS) and Co-agulase Negative Staphylococci (CONS) that isolated from different parts of human body. (79%) of isolates was COPS while (21%) was CONS. All isolates showed production some virulence factors like (Protease, DNase, Lipase, Licithinase and Haemolysin) and the results were in COPS (64, 64, 55, 55 and 100)% respectively while all isolates of CONS were produced all this virulence factors except the licithinase. The results of all isolate were positive for oxidase test and negative for catalase test. From the results about (36.3%) of COPS produce white pigment and (63.6%) produced yellow pigment while all isolates of CONS was produced white pigment. Also the results showed that (86% and 71%) COPS isolates was produced haemolysine enzyme type (β and γ) while (14% and 29%) of CONS produced same enzyme type. Antibiotic susceptibility of all isolates were done, the results showed that all CONS isolates were Susceptible towards (Amikacin, Tobramycin, Tetracline, Nitrofurantoin, Chloramphenicol and Clarithromycin) antibiotics but (33.3%) of CONS isolates were showed susceptible towards (Ampicillin, Amoxicillin, Cefdinir and Vancomycin) antibiotics. While in the COPS isolates showed low susceptibility rate towards (Vancomycin, Tetracycline Amikacin and Tobramycin) antibiotics and in different percentage (81.8, 54.5 and 90.9)% respectively while (42.8, 36.3, 9, 18.1, and 45.5)% were susceptible to (Ampicillin, Cfidor, Pencillin, Amoxicillin, Ticarcillin-clavulanic acid, Tobramycin, Cephalothin, Erythromycin, Chloramphenicol and Clarithromycin) respectively.

In conclusion, all isolates which have high resistance to antibiotics have low susceptible to same antibiotics.

Key word: Co-agulase Positive Staphylococci (COPS), Co-agulase Negative Staphylococci (CONS), virulence factors and Antibiotic susceptibility.

Introduction:

The Staphylococci are gram-positive bacteria, non-motile, not producing spores ubiquitous bacteria that include different opportunistic/pathogenic species, responsible for human and animal infections. These groups of microorganisms colonize skin, hair, nose and throat of people and animals and from these sources they can be transferred to food because both organisms are the main reservoirs. Staphylococci divide in two planes and, therefore, grow in clusters a diverse group of bacteria that cause diseases ranging from minor skin infections to life-threatening bacteraemia. They persist as a major cause of both hospital and community acquired infections worldwide. [1,2]. Staphylococcal infections are caused mainly by strains that have already colonized parts of the human body, making the colonized persons a reservoir for the spread of the organisms [3]. Detection of staphylococcal virulence factors may be a necessary step for prevention, control, and treatment of the infections. This may lead to more effective infection control practices, decreasing colonization, and development of vaccines and new or improved antimicrobial agents [4]. *S. aureus* are very adaptable pathogenic bacteria. Because of immune evasion and interference they are difficult to eliminate. Also, *S. aureus* is able to survive in almost any environment including inanimate objects for long periods [5]. The bacteria are able to multiply within the center of these abscesses without infiltrating immune cells. Eventually the lesion can rupture,

spreading even more of the pathogen into the blood circulation and to uninfected tissues [6]. However, Staphylococcus bacteria also use coagulation as an immune evasive strategy. *Staphylococcus aureus* is one of these bacteria, which produces virulence factors that enable blood clotting. This results in the formation of abscesses where *S. aureus* can replicate freely and the depletion of clotting factors from the blood [7]. Treatment of staphylococcal infections is becoming difficult due to increased antibiotic resistance [8-9]. Antimicrobial resistance is the reduction in the susceptibility of pathogenic microorganism to one or more of the chemotherapeutic agents administered in clinical medicine [10].

Materials and methods

1- Collection of Samples:

The samples under study have collected of different parts of human body from (sputum, urine, wound, abscess, burns and ear swabs). It has been selected to medical importance and causing various diseases to humans. Different media were used to growing it after diagnostic pathogenic case and growing on cultural media (Nutrient agar, Blood agar and Mannitol salt agar) then incubated at 37 C° for 24 hours.

2- Identification of isolates under study

Bacterial isolates were identified according [11,12,13,14,15,16,17,18]

A- Morphological Identification:

Isolated bacterial colonies were identified according to morphology, color and consistency on Nutrient agar medium [11].

B- Microscopic examination:

Microscopic examination was used to classify bacteria to cocci, cluster and to Gram positive bacteria on the basis of their staining with gram stain, size, shape and arrangement of cells

C- Oxidase test:

Few drop of Oxidase reagent was saturated on filter paper then part of colony from 24 hours cultures was transported by wooden stick and put on saturated filter paper, if the colony gave purple color during 10 second, this indicated a positive result.

D- Catalase test:

A drop of (3% H₂O₂) was put on a clean dry slide then a colony from 24 hours culture of bacteria was transported and mixed with this reagent. Appearance of gas bubbles means positive result. Such as *Staphylococcus* and *Micrococcus* because this bacteria product the catalase enzyme, catalase enzyme breaks down hydrogen peroxide (H₂O₂) into water and oxygen and release bubble.

E - Coagulase test:

This test was used to determine the ability of microorganisms to clot plasma, This test prepared by taking one loopful of bacterial isolates that growth from 18- 24 hours and added to 0.5 ml of human plasma, the tube was incubated at 37 °C for 30 minutes. If no clot appeared, the tube was incubated for further 24 hours, Appositive coagulase was represented by appeared of clotting.

F- Mannitol fermentation test:

Mannitol salt agar was inoculated with pure colony of isolated bacteria and incubated at 37°C for 24 hours. *S. aureus* is very tolerant of high concentrations of sodium chloride, up to 1.7 molar

G- Determination of hemolysins production:

All samples were first cultivated in Brain Heart Infusion agar and incubated at 37 °C overnight. The strains were cultured on blood agar plates, The Blood agar had a volume of (5% ml) human's blood supplemented to Blood Agar Base then cooled to 45C. Cultures incubated at 37° C for 24h. A positive result was indicated by the formation of a clear zone of haemolysis (β-haemolysis), a partial and greening haemolysis zone (α-haemolysis), or no activity (γ-haemolysis) around the spots.

H-Determination of Lipase and Lecithinase

Lipolytic activity was determined by using medium consists of: Nutrient agar, sterile (85ml) and Egg-Yolk suspension (15ml). The Nutrient agar was prepared according to the instruction of manufacturer company, sterilized by autoclaving at 121°C for 15min.then cooled to 50°C, the 15ml of Egg-Yolk suspension was added for each 85ml of sterile nutrient agar, mixed well then poured in a sterile Petri-dish. A positive result was defined by the formation of opacity around the colonies after incubation at 37°C for 24 hours. In same time we

could determination the ability of bacteria to produce lipase by dishes in cuso₄ for 20 minutes then incubated for 30min. The production of lecithinase was studied in A positive result was indicated by the formation of an opaque halo around the colonies.

I- Determination of Dnase Tests

DNase Test Agar is used for the differentiation of microorganisms on the basis of deoxyribonuclease activity. DNase agar was inoculated with an overnight broth culture of bacteria and incubated for 18-24 hours at 35°C, flood DNase Test Agar plates with 1N HCl reagent and observe for reaction. Clear area surrounding growth (band/spot inocula) on DNase Test Agar after the addition of 1N HCl indicates a positive reaction.

J- Determination of protease production:

Production was detected on skim milk agar plates, this medium consists of Nutrient agar, sterile (87.5ml) and Skimmed milk, sterile (12.5ml).The Nutrient agar was prepared according to the instruction of manufacturer company. The skim milk was sterilized by autoclaving at 121°C for 5min., cooled to 40-45°C. 12.5ml of sterile skimmed milk was added for each 87.5ml of sterile nutrient agar, mixed well then poured in a sterile Petri-dish. Casein is the predominant protein in milk, and its presence causes milk to have its characteristic white appearance, protease which hydrolyzes casein to produce more soluble peptides, transparent derivatives. The hydrolyzed clear zone around the colonies considered positive result, the results obtained by this study showed that most of the isolates were protease producers.

K- Antibiotics sensitivity test:

The antibiotic disc which are shown in table (1) were placed on the surface of Muller – Hinton agar media with a sterile forceps after spreading out the bacterial inoculums by streaking method . The cultivated plates were incubated for 24 hours at 37 °C.

4- Statistical Analysis

The data in this study were analysis according to Duncan's Multiple Range Test (DMRT) the statically test results were considered highly significant $p \leq 0.05$ no significant $p \leq 0.05$ [19] and by using Minitab program in computer.

Results and Discussion:

Staphylococcus has been considered to be a major public health issue because it can cause both health-associated and community -associated infections, with considerable morbidity and even mortality. The infections are either minor such as infections of skin and soft tissues or more serious systemic infections including endocarditis, osteomyelitis, and septic shock syndrome [20]. So we studied some characters and virulence factors of some *Staphylococci* species which causing different disease of human. In this study the specimens had taken from different sites of human body. Gram stain was done to the all isolates then Microscopic examination was applied to the isolates, the cells appeared as Gram-

positive cocci arranged in grape-like irregular clusters. Isolates cultured on Mannitol salt agar which considered selective and differential media for genus *Staphylococcus* [21] The colonies appeared round, smooth, raised, mucoid and glistening. Consequently, the isolates belong to the genus *Staphylococcus*. Some isolates had the ability to ferment mannitol and form large golden colonies surrounded by wide yellow zones and turned the colour of the medium from pink to yellow. The coagulase test was done to all isolates, from the (79%) of isolates showed the ability to produce coagulase enzyme (coagulase-positive) (COPS) while (21%) were coagulase-negative (CONS) (Figure 1). In addition to catalase test was performed and the test was positive, all isolates appeared ability to ferment mannitol and gave positive results. The oxidase test was also done and isolates gave negative result [16,17,22].

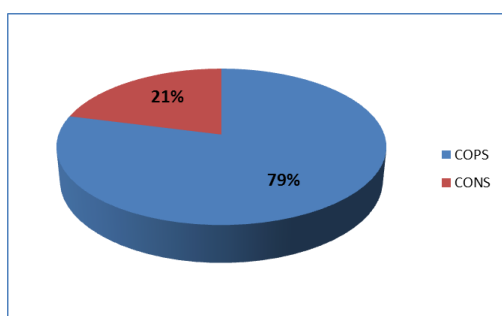


Figure (1) the percentage of Co-agulase Positive Staphylococci (COPS) and Co-agulase Negative Staphylococci.

The isolates were tested for their ability to produce a variety of pigments on the skimmed-milk agar (Fig.1) and the colonies varied from white to very deep golden yellow Table (1).

Table (1) the percentage of pigment production

Pigment type	Bacterial isolates	
	COPS (%)	CONS (%)
White	36.3	100
Yellow	63.6	0

Pigments of various colors are synthesized to protect the cells of micro-organisms from injurious effect of light rays of visible and near ultraviolet range [23]. These pigments are synthesized by various types of microorganisms as secondary metabolites and not often found in all types of organisms [24]. The golden pigmentation of *S. aureus* colonies is caused by the presence of carotenoids and has been reported to be a virulence factor protecting the pathogen against oxidants produced by the immune system [25,26] There is growing interest in microbial pigments due to their natural character and safety to use, medicinal properties, nutrients like vitamins, production being independent of season and geographical conditions, and controllable and predictable yield [27]. Again microbial pigments can be produced from waste material reducing water and environmental pollution [28].

Table (2): The percentage of the virulence factors of COPS and CONS

Virulence Factors	COPS Isolates		CONS Isolates	
	Positive %	Negative %	Positive %	Negative %
Protease	64	36	100	0
Lipase	55	45	100	0
DNase	64	36	100	0
licithinase	55	45	0	100
Haemolysins	100	0	100	0

From the results appears in (Table 2)(Fig. 1) the high level of protease and DNase production (Fig. 4) in both COPS and CONS isolates in the same rate of production reach to 64% in COPS and 100% in CONS isolates. This result was agreed with the study by [29] who found that (65.9%) CONS and (26.6%) COPS demonstrated protease activity. Also this result agreed with the finding of [30] who reported that 50% of CONS produce protease. The rate of lipase production (Fig.2) was (55%) in COPS isolates while (100%) of CONS isolates were produced this enzyme, This result agreed with the study of [31] who found *Staphylococcus aureus* that were isolated from the deep wound infections produced higher amounts of lipase On the other hand our results disagreed with the study of [30] who found that 68.1% of CONS isolates produce this enzyme.

Rooijackers and others founds that coagulase negative staphylococci isolated from acne lesions were demonstrated to present lipase and protease activities more often than coagulase positive staphylococci. The potent serine protease of plasmin is activated and cleaves surface-bound C3b and IgG, resulting in reduced phagocytosis by neutrophils. Lipase activity might be important for nutrition or dissemination of the bacteria. The strongest hint that was ever found out about the correlation between lipase activity and pathogenesis of staphylococci is the detection of anti-lipase IgG antibodies in patients with the *Staphylococcus aureus* infections which showed the pathogenetic potential of the extracellular lipase [33]. Two secreted lipases support the colonization and growth of the bacteria by the cleavage of the triacylglycerols derived from the sebum of the skin [34].

Lethicinase production (Fig.2) by the isolates also tested in both COPS and CONS and the result indicated a significant difference between the two groups of isolates. 55% COPS isolates were found produce lethicinase while all CONS isolates were not produced this enzyme. *S. aureus* secrete several extracellular enzymes whose function is thought to be the disruption of host tissues and/or inactivation of host antimicrobial mechanisms (e.g. lipids, defensins, antibodies and complement mediators) to acquire nutrients for bacterial growth and facilitate bacterial dissemination [35,36].

Table (3) the percentage of Haemolysin production to both COPS and CONS

Haemolysin type	Bacterial isolates	
	COPS(%)	CONS(%)
β	86	14
γ	71	29

Finally, Haemolysin test also done and we found significant differences, both CONS and COPS were produced Haemolysin enzyme (Fig.5) in two type (β and γ Haemolysis) but the rate in COPS was higher than from CONS, it reach to (86% and 71%) respectively while in CONS the rate was (14% and 29%) respectively, These results agreed with the study of [36] who found that all *S. aureus* tested isolates produced β -haemolysin and disagree with [37] who demonstrated that 80.9% of CONS isolates gave haemolytic activity.

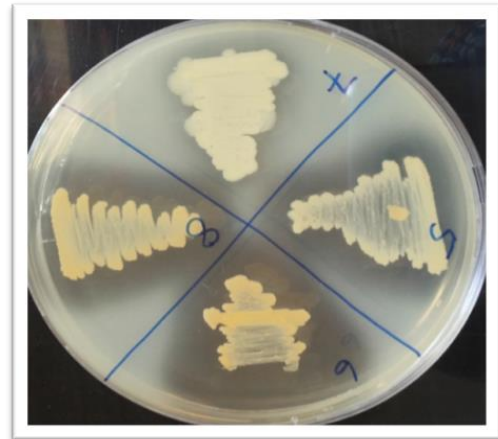


Fig.(4): The DNase production (clear zone around colonies)



Fig.(2): Types pigments and protease production (clear zone) around colonies on Skim-milk agar

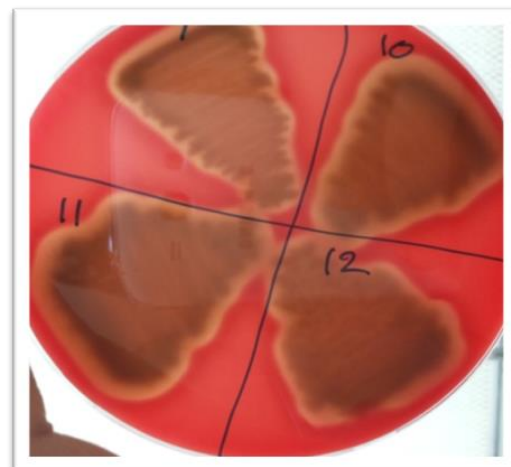


Fig.(5): The Haemolysin production



Fig.(3): Lipase production (clear zone) Lecithinase production (white zone) On Egg - yolk agar

Table (4) the Susceptibility of COPS and CONS isolates to antimicrobial agents.

Antibiotics/discs concentration	CONS			COPS		
	R(%)	S(%)	I(%)	R(%)	S(%)	I(%)
Ampicillin 10 µg	66.6	33.3	0	63	42.8	14.2
Oxacillin 1 µg	100	0	0	100	0	0
Pencillin 10U	100	0	0	63	36.3	0
Amoxicillin/clavulanic acid 20/10 µg	33.3	33.3	33.3	81.8	36.3	9
Ticarcillin/clavulanic acid 75/10 µg	100	0	0	90.9	9	0
Cephalothin 30 µg	100	0	0	81.8	18.8	0
Ceftriaxone 30 µg	100	0	0	100	0	0
Cefaclor 30 µg	0	0	100	0	42.8	72.7
Cefdinir 5 µg	0	33.3	66.6	45.4	9	45.4
Vancomycin 30 µg	33.3	33.3	33.3	18.1	81.8	0
Amikacin 30 µg	0	100	0	36.3	54.5	9
Tobramycin 10 µg	0	100	0	9	90.9	0
Clarithromycin 15 µg	0	100	0	45.4	45.4	9
Erythromycin 15 µg	0	100	0	45.4	45.4	9
Tetracycline 30 µg	0	100	0	18.1	81.8	0
Nitrofurantoin 300 µg	0	100	0	0	100	0
Trimethoprim 5 µg	100	0	0	100	0	0
Chloramphenicol 30 µg	0	100	0	45.4	45.4	9

Antimicrobial susceptibility test was done against all bacterial isolate. 18 antibiotics that appeared in Table(1) were applied on all isolates included both COPS and CONS. The susceptibility test was performed according to the Kirby-Bauer Method (antibiotic disc diffusion method).

From the results that appeared in table to both COPS and CONS isolates showed all isolates (100%) were resistant to Oxacillin, Trimethoprim and Ceftriaxone antibiotics. The coagulase-positive and negative strains resistance to Ampicillin antibiotic with a few differences and variation in the rates of resistance the rate resistance of COPS to the Ampicillin was 63% while the rate of CONS resistance was 66% and this result agreed with many studies that demonstrated the increasing in the rate of sulfamethazole-trimethoprim resistance is probably due to continuous use of it for many years, so the long exposure of bacteria to this antimicrobial agent through uses [38]. The widespread use and more often the misuse of antimicrobial drugs has led to a general rise in the emergence of resistant bacteria [39]. and our study was agreed with [40] were found that *Staph. Aureus* little low effect and completely resistance to oxacillin.

The lowest rate resistance of CONS was towards antibiotics Amoxicillin/ Clavulanic and Vancomycin in same rate 33.3% this result disagreed with [41] they found that both CONS was fully susceptible to the Vancomycin (100%). And in another study by [42] found that all Staphylococci (*S.aureus* and CONS) remained fully susceptible to Glycopeptides antibiotics like Vancomycin and Teicoplanin, but after that [43] mentioned that although the remaining effective therapy against most strains of multidrug-resistant Staphylococci is the Glycopeptide antibiotic Vancomycin. Also COPS showed resistance to others

antibiotics like Amoxicillin/Clavulanic acid, Cephalothin and Ticarcillin-clavulanic acid the rate of resistance was 63%, 81.8%, 90.9% respectively. Beta-lactam antibiotics, the most effective and widely used class of antimicrobials. Moreover, in many countries clinical strains are quite often multi-resistant which significantly reduces the therapeutic options for treatment of staphylococcal infections [44]. From the results we showed the decreased in resistance to Cefdinir, Vancomycin.

The results showed 100% susceptibility of CONS toward Amikacin, Tobramycin, Tetracycline, Clarithromycin and Chloramphenicol while in COPS was also susceptible to Amikacin, Tobramycin, Tetracycline antibiotics but in different rates, this rates was 81.8%, 54.5%, 90.9%, 81.8% respectively, aminoglycosides inhibition protein synthesis, in all cells, protein synthesis requires not only the information stored in DNA plus several kinds of RNA but also ribosomes. Differences between bacterial (70S) and animal (80S) ribosomes allow antimicrobial agents to attack bacterial cells without significantly damaging animal cells that is with selective toxicity. Amikacin is especially effective in treating hospital acquired infections resistant to other drugs, it should not be used in less demanding situation lest organisms become resistant to it, too. Chloramphenicol and erythromycin act on 50S portion of bacterial ribosomes, inhibiting the formation of the growing polypeptide. Because animal cell ribosomes consist of 60S and 40S subunit [45].

The susceptible to nitrofurantoin in COPS and CONS was 100% while the resistance of COPS was decreased toward Clarithromycin and Chloramphenicol antibiotics the rate was similar in both antibiotic it was 45.4 and this result disagreed with [40] were found *S. aureus* was highly sensitive

to Nitrofurantoin (100%). The low level of resistance to Nitrofurantoin among uropathogens at approximately 2%, it remains an ideal therapeutic agent [46,47]. So recommended Nitrofurantoin as

drug of choice for the immediate empirical therapy[48].

Finally we showed that all isolates which have high resistance to antibiotics have low susceptible to same antibiotics.

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دراسة بعض عوامل الضراوة لبكتريا المنتجة و غير المنتجة للإنزيم المخثر للبلازما Staphylococci المعزولة من مناطق مختلفة من جسم الانسان

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الملخص

تضمنت هذه الدراسة اختبار انتاج بعض عوامل الضراوة المنتجة لدى بعض انواع بكتريا Staphylococci: المنتجة لانزيم التجلط (COPS) وغير المنتجة لانزيم التجلط (CONS) المعزولة من مناطق مختلفة من جسم الانسان حيث كانت النتيجة 79% من العزلات منتجة لانزيم التجلط بينما 21% كانت سالبة لانزيم التجلط. ان نتائج جميع العزلات كانت موجبة لاختبار الاوكسيديز وسالبة لاختبار الكاتاليز. بينت النتائج بان جميع العزلات كانت منتجة لبعض عوامل الضراوة وينسب مختلفة مثل (البروتيز ، DNase، اللايباز ، الليسيثينز والهيمولايسين) كانت النسب عند COPS هي (64،64، 55، 55، 100)% على التوالي بينما كانت جميع عزلات CONS منتجة لجميع عوامل الطراوة المذكورة باستثناء انزيم الليسيثينز. اوضحت النتائج ان حوالي 36.3% من COPS انتجت صبغة بيضاء بينما 63.6% منها انتجت صبغة صفراء اما بالنسبة لـ CONS فقد اظهرت جميعها صبغة بيضاء. كذلك بينت النتائج ان COPS كانت منتجة لنوعين من الانزيمات الحالة للدم (β 86% و γ 71%) بينما CONS فقد اظهرت (β 14% و γ 29%). تم اجراء فحص الحساسية لجميع العزلات البكتيرية قيد الدراسة حيث اظهرت النتائج ان جميع عزلات CONS كانت حساسة لمضادات (Amikacin, Tobramycin, Tetracline, Nitrofurantoin, Chloramphenicol) لكن (Clarithromycin) لكن 33.3% كانت حساسة تجاه (Vancomycin و Ampicillin, Amoxicillin, Cefdinir) بينما عند عزلات COPS اظهرت حساسية اقل تجاه (Tobramycin و Vancomycin Amikacin, Tetracycline,) وينسب مختلفة (81.8، 54.5، 90.9) % على التوالي بينما (42.8، 36.3، 9، 18.1، 45.5) % كانت حساسة للمضادات (Ampicillin, Cfidor, Pencillin, Amoxicillin, Ticarcillin-clavulanic acid, Tobramycin, Cephalothin, Erythromycin, Chloramphenicol, Clarithromycin) على التوالي. تم الاستنتاج ان البكتريا ذات المقاومة العالية للمضادات الحيوية امتلكت حساسية اقل تجاه نفس المضادات.

الكلمات المفتاحية: بكتريا Staphylococci: المنتجة لانزيم التجلط (COPS) ، غير المنتجة لانزيم التجلط (CONS)، عوامل الضراوة والحساسية للمضادات الحيوية.