PRELIMINARY CHARACTERIZATION AND IDENTIFICATION OF GRAM POSITIVE HEMOLYSIS BACTERIA

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ABSTRACT:

One of the most important pathogens that threaten human health all over the world is *Staphylococcus* spp. characterization and identification of such pathogen considers a useful tool to control of some serious problems resulting by these bacteria. Therefore one hundred and twenty samples including blood, urine, abscess, semen, pus, sputum, ears, vaginal and spit swabs were collected from patients of Tanta University Hospital and outpatient clinics. A total of 126 Gram positive bacterial isolates were obtained from these clinical specimens. Of these isolates 107 bacterial strains were identified as *Staphylococcus* spp and 15 strains identified as *Streptococcus* spp in addition to 4 strains were identified as *Enterococcus* spp, preliminary identification was conducted on the basis of colony characteristics such as Gram staining, pigment production, hemolysis, catalase activity, coagulase test and fermentation of manitol sugar. Out of these strains, 30.95% have the potency to make alpha hemolysis while 30.95% have the ability to make beta hemolysis and 38.09% posse the capacity to make gamma hemolysis on blood agar medium. Beta hemolysis *Staphylococcus* spp were selected for study of some virulence factors on basis of coagulase production in which 17.24% of *Staphylococcus* spp were coagulase positive and 82.76% were coagulase negative *Staphylococcus* spp. Studying the susceptibility pattern of these strains to some commercial antibiotics was carried out. Further future studies are recommended to investigate the effect of some natural compounds on gene regulation that responsible for hemolysis process.

Keywords: Hemolysis- Gram positive bacteria- *Staphylococcus*- Coagulase production

Introduction

One of the most important Gram positive medical bacteria is *Staphylococcus aureus.* Its opportunistic pathogens for human and animal responsible for fatal disease like nosocomial and community-associated infections which make high morbidity and mortality rate. *S. aureus* can cause diseases ranging from soft tissue infections and minor skin to life-threatening invasive diseases such as osteomyelitis, endocarditis, septicemia, and pneumonia (**Al-mebairik *et al.*, 2016).**

S. aureus attachment to tissue then invading it as well as avoiding host immune response through its virulence factors **(Gnanamani *et al*., 2017).** The difference of site of infection by *S aureus* tend to the diversity of virulence factors and its ability to adaptation to different environments in the human host **(Tuchscherr *et al*., 2019).**

S. aureus appear as large yellow or white colonies on nutrient rich agar media. This pathogen can produce carotenoid pigments which imparts the yellow color on its colonies, so the term *aureus* refers to Latin word the color of gold. This yellow pigments act as antioxidant that protect this strain from the host's immune system. The organism also has the ability to produce different type of hemolysis like alpha, beta, gamma and delta.Several exotoxins can be secreted by this organism like coagulase , enterotoxins, toxic shock syndrome toxin-1 (TSST-1), and protein A. The medical importance of this strain is due to production of virulence factors, surface proteins, enzymes, toxins and biofilm formation in addition to rapid development of drug resistance **(Abu-elghait 2016)-**Bacterial resistance to antibiotics is an issue that has led to the search for new antibacterial approaches. Drugs targeting virulence is an alternative approach to treat infections with resistant bacteria **(Escaich, 2008).**

The ability of its transferring from person to surrounding area or another person makes it widespread pathogen **(Michael & Roberts, 2016).** Therefore, billions of dollars are consuming every year by several countries such as the United States to control the infections that caused by this pathogen **(Al-mebairik. 2016).** Hence this study is aimed to isolation, characterization and identification of some hemolytic gram positive bacteria specially *S. aureus* from different localities of Egypt.

MATERIALS AND METHODS

Samples collection

A total of 120 samples were collected from different clinical specimens as the following ; blood, abscess, pus, sputum, urine, wound swabs, vaginal swabs and cerebral spinal fluid from inpatient and outpatient of Tanta University Hospital through November 2017 to march 2018**(Karmakar *et al*., 2015)(Biswas *et al*.,2016)**

Trypticase Soy Agar (TSA)

This media is used for cultivation and isolation of fastidious and nonfastidious microorganisms. This medium is composed of pancreatic digest of casein (a peptone) 15 g\l , enzymatic digest of soybean 5g\l, sodium chloride 5 g\l, agar 15.0g\l and finally pH was adjusted at 7.3 ± 0.2 (**Orth *et al.,* 1993),(Curry *et al.*,1993).**

Blood Agar medium

Blood agar medium is a highly enriched medium so it preferred for fastidious organisms and also often used to distinguish between pathogenic bacteria via determining the presence or absence of hemolysis and its type on red blood cells **(Turista *et al.,* 2019).** The composition of this medium is peptone10g/l, Tryptose10g/l, Sodium chloride 5g/l, Agar15g/l and pH was adjusted at 7.3± 0.2. Preparation of this medium is by adding 40 gram of dehydrated medium to 1liter of distilled water then autoclaving for15 min at 121℃ after that let it to cool to 45℃ and add 5% v/v sterile defibrinated sheep blood or human blood to sterile media then mix vigorously **(Yeh *et al.,* 2009).**

Manitol Salt Agar (MSA)

Manitol Salt Agar considers as an excellent medium for isolating staphylococci from heavy contaminated cultures in which coagulase positive *staphylococci* appear on this medium as a yellow colonies with yellow zone and coagulase negative *staphylococci* appear as a small red colonies without any change of the color of the medium while *Micrococcus* colonies will seem as a white to orange colonies and also with no change in medium color, on the other side any different strain will not grow on MSA.The MSA is consist of; pancreatic digest of casein 5.0 g/l, Peptic Digest of Animal Tissue 5.0 g/l, beef extract 1.0 g/l, sodium chloride 75.0 g/l, D-mannitol10.0 g/l, phenol red 25.0 mg/l and agar15.0 g/l **(Interim *et al.,* 2016)**

Mueller Hinton Agar and Antibiotic susceptibility assay

This medium is ideal for antibiotic susceptibility test (disk diffusion method). Its composed of Beef Extract 2.0 g/l, acid hydrolysate of casein 17.5 g/l, starch 1.5 g/L and agar 17.0 g/l. Final pH should be adjusted at 7.3 ± 0.3 **(Mueller *et al.,* 1941), (Washington *et al.,* 1995)**,**(Clinical and Laboratory Standards Institute, 2009).**

the susceptibility to specified antibiotic is determined by measuring the clear zone around its disc on the plate**.** The result falls into three categories; resistant, intermediate, and susceptible**.** The antibiotics were used in our study were gentamicin (10 µg), erythromycin (15 µg), oxacillin (1 µg), ciprofloxacin (5 µg), ampicillin (10 µg), linezolid (30 µg) clindamycin (2 µg), vancomycin (30 µg), teicoplanin, (30 µg) and doxycycline (30 µg) **(Kulkarni *et al.,* 2019),(Garg *et al.,* 2019).**

Phenotypic Identification of *Staphylococcus* spp.

The identification of *Staphylococcus* strains were performed on the basis of Standard microbiological methods including colony characteristics, Gram staining,

pigment production, hemolysis and the following biochemical reactions, catalase activity, coagulase test (human plasma) and manitol fermentation, finally the most potent isolates was identifying by a full biochemical tests through BIOMERIEUX VITEK2 SYSTEM **(Desouky *et al*., 2014).**

Catalase test

Catalase test was conducted to detect the ability of bacteria to produce catalase enzyme through three common methods; the slide test method, the tube or bottle method and the agar slant method, during this test slide tube method was applied by adding 1 drop of 3% H_2O_2, theoretically the catalase enzyme will accelerate the breakdown the hydrogen peroxide into water and oxygen **(Reine& Karen, 2010) .**

Oxidase test

This test was applied on a catalase positive isolates to detect the capability of these isolates to secrete oxidase enzyme. This test theoretically based on the presence of indophenol oxidase enzyme which catalyzes the electron transportation from donor compounds (NADH) to electron acceptors (usually oxygen) **(Shields *et al*,. 2016) .**

Coagulase Test

This test was performed to detect the presence of coagulase enzyme which able to convert plasma fibrinogen to fibrin causing agglutinates or a clot. There are two types of coagulase; bound coagulase which is bounded to cell wall and free coagulase which is liberated by cell wall. This test used mainly to distinguish *S. aureus* strains from other different *Staphylococcus* strains **(Desouky *et al*., 2014).**

Hemolysin Production

Qualitative hemolysis assay were completed on blood base agar medium supplemented with 5% sheep blood and the samples were one of three forms, the first form was a clear zone around the colonies which distinguished by its transparency because a complete lysis of red blood cells, these isolates recorded as a β hemolytic isolates. The second form was a brownish green color zone around colonies and this discoloration of the medium color was because the isolates could reduce the hemoglobin of red blood cells to methemoglobin which distinguished by a brownish green color, these isolates was recorded as α hemolytic isolates. The third form was no change in medium color so these isolates recoded as a γ hemolytic isolates **(Buxton & Rebecca 2016), (Divyakolu *et al*,. 2019).**

Quantitative hemolysis assay were applied according to Cho and his co-team on only a coagulase, catalase and manitol salt fermentation positive isolates which recorded as a β hemolytic isolates and distinguished with a cluster shape under microscope **(Cho *et al*., 2015).** *S. aureus* culture were diluted at 1:100 with TSB culture media and incubated at 37℃ for 16 hours under shaking condition at 250 rpm. Human red blood cells separated by centrifugation at 900xg for 5 min, then human red blood cells washed three times with PBS buffer and diluted at 3% of red blood cells in PBS buffer, after

that the cell culture 50µ were added, the mixture were incubated under shaking condition at 250rpm at 37℃ for 1 hour. *S. aureus* (ATCC 29213) were used as a positive control and the negative control inoculated with PBS buffer instead of the organism. the optical density was measured at 543 nm after supernatant collection by centrifugation at 16,600xg for 10 min **(Guo *et al*., 2019),(Tang *et al*., 2019).**

RESULTS AND DISCUSSION

A total of 126 gram positive bacterial isolates were obtained from several clinical specimens that collected from different sources. Of these isolates 107 (85%) bacterial strains were preliminary identified as *Staphylococcus* spp and 15 (12%) strains identified as *Streptococcus* spp in addition to 4 (3%) strains were identified as *Enterococcus* spp. The higher percent of *Staphylococcus* spp. occurrence that isolated from clinical samples indicates on the higher transition rate of this bacteria hospital community and different medical tools and this reflected the importance of studying of this type of microorganism as described by **(Desouky *et al*., 2014)**.

Bacterial identification was conducted on the basis of colony characteristics such as gram staining, pigment production, hemolysis, catalase activity, coagulase test and fermentation of manitol sugar. Gram stain is used to help in differentiating between gram positive cocci strains. All bacteria isolated during this study are gram positive, hence the cell arrangement is required to differentiate between these genera in which some bacteria like staphylococci commonly divide on random planes to form grapelike clusters shape while other divide in one plane form pairs or chains and this arrangement commonly observed by *Streptococcus* and *Enterococcus*. As well as catalase and oxidase activity are aid in differentiating between gram positive genera because *Streptococcus* and *Enterococcus* are negative for both testes while *Staphylococcus* spp. are positive for the same testes.The highest number of *Staphylococcus* strains were isolated from female which represented as 12 strains (41.38%) and then from male represented as 10 strains (34.48%), on the other hand the lowest number of strains were founded in babies and child and represented as 6 (20.69%) and 1 (3.45%), respectively as showed in Fig1 and this may be refers to that females are the most type exhibited to *Staphylococcus* spp. risks.

Staphylococcus strains were collected from several clinical specimens and the higher number of this strain was isolated from automated blood cultures and then from sputum and finally from urine with percentage 48.28% (14 strains), 17.24% (5 strains) and 13.79% (4 strains), respectively, while the lower number of this strain was collected from pus, trachea, manual blood cultures, ascetic fluid cultures, Pleural fluid with ratio 6.91% (2 strains) and 3.45% (1strain) for the other sources respectively as represented in Fig 2. The predominance of the isolation *Staphylococcus* strains from blood cultures indicates the need for the more bacteriological examination in this source, because of the infection that may by cause by these strains and reach to this source.

In the present study some virulence factors are studied which consider as correlated tools to pathogenicity caused by this bacteria as noted by **(Koneman *et al*., 1988), (Otto, 2004), (Lee *et al*., 2012)**. In which 30.95% (39 strains) have the potency to make alpha hemolysis while 30.95% (39 strains) have the ability to make beta hemolysis and

38.09% (48 strains) posse the capacity to make gamma hemolysis on blood agar medium.

The pathogenesis of *S. aureus* infections depends on the production of surface proteins that mediate bacterial adherence to host tissues, secretion of a series of extracellular toxins, and enzymes that destruct host cells and tissues, avoidance of, or incapacitating, the host immune defense, and growth and spread of bacteria in host cells as mention by **(Lowy, 1998).** Add to that the most important virulence factor in Staphylococcus spp. is hemolysins that damage the host cells by making pores in it as recorded by **(Dinges *et al.*, 2000).** *Staphylococcus* strains were selected for study of some virulence factors based on their coagulase activity which differentiated into 5 isolates (17.24%) were *Staphylococcus* spp. coagulase positive while 24 isolates (82.76%) were coagulase negative *Staphylococcus* spp. Primary description of these isolates was illustrated in table 1 and their coagulase activity was showed in Fig 3.

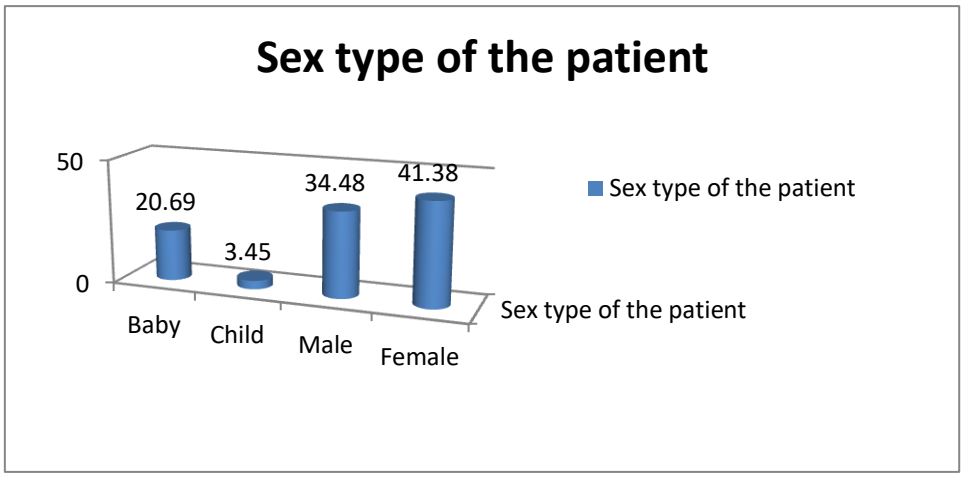

Fig 1: Percentage of *Staphylococcus* strains related to different patient sex.

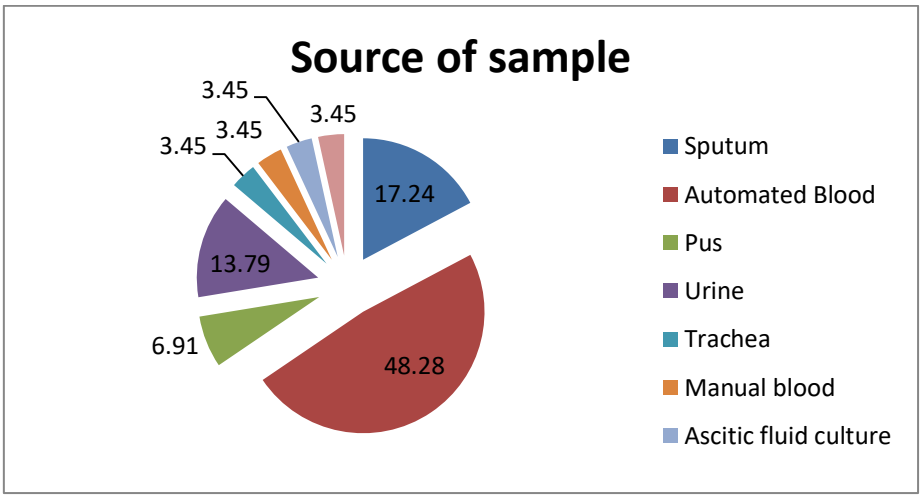

Fig 2: Percentage of *Staphylococcus* strains related to different sample collection sources.

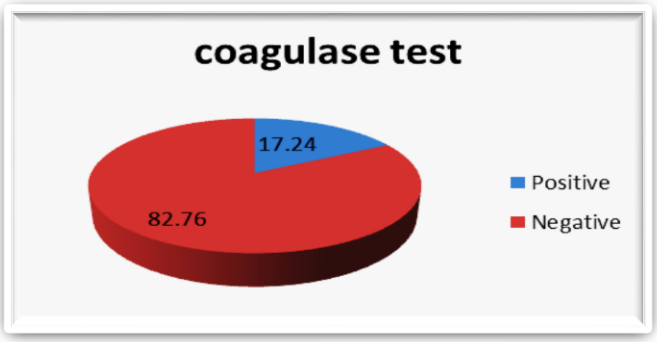

Fig3: Percentage of coagulase positive and coagulase negative between *Staphylococcus* spp. Isolated from different source and sex.

Table 1: primary description of *Staphylococcus* spp. strains from the collected samples related to their sources and sex

Sample code	Sex type	Sample source	Color on MSA	Coagulase test	Catalase test	Gram stain/shape
I1	M	Spit culture	Yellow	Negative	Positive	+ve cocci
I58	M	Sputum	Yellow	Negative	Positive	+ve cocci
I76	M	Blood	Yellow	Negative	Positive	+ve cocci
O41	Baby	Blood	Red- Yellow	Negative	Positive	+ve cocci
O80	F	Blood	Yellow	Negative	Positive	+ve cocci
I73	F	Sputum	Yellow	Negative	Positive	+ve cocci
O93	F	Pus	Yellow	Positive	Positive	+ve cocci
I111	M	Trachea	Yellow	Negative	Positive	+ve cocci
O46	M	Urine	Yellow	Negative	Positive	+ve cocci
I49	M	Urine	Yellow	Positive	Positive	+ve cocci
I103	M	Urine	Red- Yellow	Negative	Positive	+ve cocci
O40	F	Blood	Red- Yellow	Negative	Positive	+ve cocci
O64	Baby	Blood	Yellow	Negative	Positive	+ve cocci
I82	F	Sputum	Yellow	Positive	Positive	+ve cocci
I104	F	Sputum	Yellow	Negative	Positive	+ve cocci
I53	Baby	Blood	Yellow	Positive	Positive	+ve cocci
O75	F	Blood	Yellow	Negative	Positive	+ve cocci
O88	M	Blood	Yellow	Negative	Positive	+ve cocci
O15	F	Urine	Yellow	Negative	Positive	+ve cocci
O54	Child	Pus	Yellow	Negative	Positive	+ve cocci
O83	F	Blood	Yellow	Negative	Positive	+ve cocci
O44	M	Blood	Red- Yellow	Negative	Positive	+ve cocci
O85	Baby	Blood	Yellow	Negative	Positive	+ve cocci
O114	F	Blood	Yellow	Negative	Positive	+ve cocci
O79	F	Blood	Yellow	Negative	Positive	+ve cocci
O122	Baby	Blood	Yellow	Positive	Positive	+ve cocci
I52	F	Ascetic fluid	Yellow	Negative	Positive	+ve cocci
O126	Baby	Blood	Red- Yellow	Negative	Positive	+ve cocci
O66	M	pleural fluid	Yellow	Negative	Positive	+ve cocci

(MSA) Manitol salt agar;(+ve) positive; (-ve) negative; (F) female;(M) male

The most potent strains are selected based on the ability of strain to make complete blood hemolysis (beta hemolysins) through produce an important virulence factor α hemolysin protein with different degrees detected quantitatively by spectrophotometer 543nm as described by **(Desouky *et al.*, 2014)** by using *S. aureus* ATCC 29213 as positive control, clinical isolate *S. aureus* I82 and *S. aureus* O122 exhibit the most hemolysin producing strains as observed in table 2 and Fig 4. Strain I82 produced hemolysin near three times approximately while strain O122 produced one and half time than positive control strain, therefore both *S. aureus* strains that used in this study exhibited more hemolysin efficiency than any strains reported by Desouky and his team during quantitative detection of hemolysin production by threeteen *S. aureus* strains **(Desouky *et al.*, 2014)**

Table 2: Hemolysis degree for most potent strains producing hemolysis by quantitative method

Sample No.	O.D: 543nm	Relative hemolysis %
+ Ve	1.069	100
- Ve	0.052	4.86
82	3.010	281.57
122	1.781	166.6

O.D; Optical Density, P; Positive, N; Negative

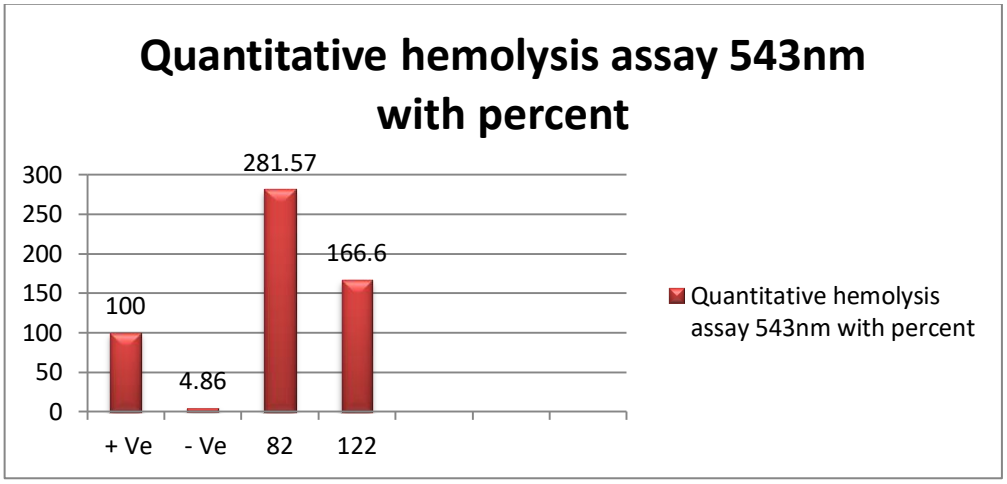

Fig 4: The quantitative hemolysis assay for the most potent strains

Identification of the most potent isolates I82 and O122 were accomplished by BIOMERIEUX VITEK2 SYSTEM in which the isolated I82 was correlated with strain *Staphylococcus aureus* that has bio-number (010402026632231) with high similarity reached to 94% and hence this strain identified as *staphylococcus aureus* I82 while the other strain O122 was related to *staphylococcus warneri* strain that has bio-number (010002073661031) with similarity 90% and hence this strain well known as *Staphylococcus warneri* O122. All morphological, physiological and biochemical characterizations recorded by VITEK2 SYSTEM are shown in table 3.

Table 3: morphological, physiological and biochemical testes for the most potent strains

Characterization			**Strains**	
Morphological characteristics			I82	O122
Color			Golden yellow	Golden yellow
Gram-reaction			+	+
Catalase			+	+
Coagulase plasma reaction			+	+
Biochemical characteristics				
D-Amygdalin Phosphatidylinositol (AMY)			–	–
phospholipase C	PIPLC		–	–
D-xylose	dXYL		–	–
Arginine dihydrolase I	ADH I		+	+
Beta-Galactosidase	BGAL		–	–
Alpha-Glucosidase	AGLU		–	–
Ala-Phe-Pro Arylamidase	APPA		–	–
Cyclodextrin	CDEX		–	–
L-Aspartate Arylamidase	AspA		–	–
Beta-Galactopyranosidase	BGAR		–	–
Alpha-Mannosidase	AMAN		–	–
Phosphatase	PHOS		–	+
Leucine ARYLAMIDASE	LeuA		–	–
L-Proline ARYLAMIDASE	ProA		–	–
BETA GLUCURONIDASE	BGURr		–	–
ALPHA-GALACTOSIDASE	L-Pyrrolidonyl	(AGAL)	–	–
ARYLAMIDASE	PyrA		+	+
BETA-GLUCURONIDASE	BGUR		–	–
Alanine ARYLAMIDASE	AlaA		–	–
Tyrosine ARYLAMIDASE	TyrA		–	–
D-SORBITOL	dSOR		–	–
UREASE	URE		+	+
POLYMIXIN B RESISTANCE	POLYB		+	+
D-GALACTOSE			+	+
D-RIBOSE	dRIB		–	+
L-LACTATE alkalinization	ILATk		+	+
LACTOSE	LAC		–	–
D-MALTOSE	dMAL		+	+
BACITRACIN RESISTANCE	BACI		+	+
NOVOBIOCIN RESISTANCE	NOVO		–	–
GROWTH IN 6.5% Nacl	NC6.5		+	+
D-MANNITOL	dMAN		+	+
D-MANNOSE METHYL-B-D	(dMNE)		+	+
Continue				
GLUCOPYRANOSIDE	MBdG		+	–
PULLULAN	PUL		–	–
D-RAFFINOSE	dRAF		–	–
O/129 RESISTANCE	O129R		+	–
SALICIN	SAL		+	+
SACCHAROSE/SUCROSE	SAC		+	+
D-TREHALOSE	dTRE		–	–
ARGININE DIHYDrolase 2	ADH2s			
OPTOCHIN RESISTANCE			+	+

+: POSTIVE Result, –;Negative Result

The antibiotic susceptibility test for the most potent isolates was conducted with different commercial antibiotics agents such as gentamicin (10μg), erythromycin (15μg), oxacillin (1μg), ciprofloxacin (5 μg), ampicillin (10 μg), linezolid (30 μg), clindamycin (2 μg), vancomycin (30 μg), teicoplanin (30 μg) and doxycycline (30 μg)

as represented in table 4.The clinical efficacy of antibacterial agents including bacteriostatic or bactericidal effects that used for the treatment of *S. aureus* infections should depend on the ability of this antibiotic to prevent the virulence factors produced by such bacteria. For the management of toxic *S. aureus* infections, some antibiotics display an anti-virulence activity at different concentrations. For instance, protein synthesis-suppressing antibiotics, such as clindamycin and linezolid, are recommended for the treatment of *S. aureus*-produced toxic syndromes, as concentrations below the MIC have been shown to impair the expression of *S. aureus* virulence factors **(Herbert *et al*., 2001), (*Bernardo et al.*, 2004).**

Table 4: Antibiotics susceptibility test for the most potent isolates

Antibiotics	**Abbreviation**	***S. aureus* I82**	***S. warneri* O122**
Gentamicin (10µg)	CN10	R	R
Erythromycin (15µg)	E15	R	S
Oxacillin (1µg)	OX1	I	R
Ciprofloxacin (5 µg)	CIP5	I	R
Ampicillin (10 µg)	SAM20	R	R
Lnezolid (30 µg)	LZD-30	S	R
Clindamycin (2 µg)	DA2	S	R
Vancomycin (30 µg)	VA30	S	S
Teicoplanin (30 µg)	TEC30	S	R
Doxycycline (30 µg)	DO30	R	I

S; Sensitive, R; Resistant, I; Intermediate

Strain *S. aureus* I82 was showed the sensitivity toward 4 (40%) of commercial antibiotics under test including linezolid (30 µg), clindamycin (2 µg), vancomycin (30 µg) and teicoplanin (30 µg) while the resistance was toward 4 (40%) antibiotics represent in ampicillin (10 µg), gentamicin (10µg), erythromycin (15µg) and doxycycline (30 µg), finally strain *S. aureus* I82 was given intermediate response to oxacillin (1µg) and ciprofloxacin (5 µg). On the contrary *S. warneri* O122 strain was showed 70% (7) resistance for this antibiotics including gentamicin (10µg), oxacillin (1µg), ciprofloxacin (5 µg), ampicillin (10 µg), linezolid (30 µg), clindamycin (2 µg) and teicoplanin (30 µg), also this strain showed intermediate response to one antibiotcs (10%) doxycycline (30 µg) while the sensitivity was founded toward 20% (2) erythromycin (15µg) and vancomycin (30 µg) as represent in Fig 5.

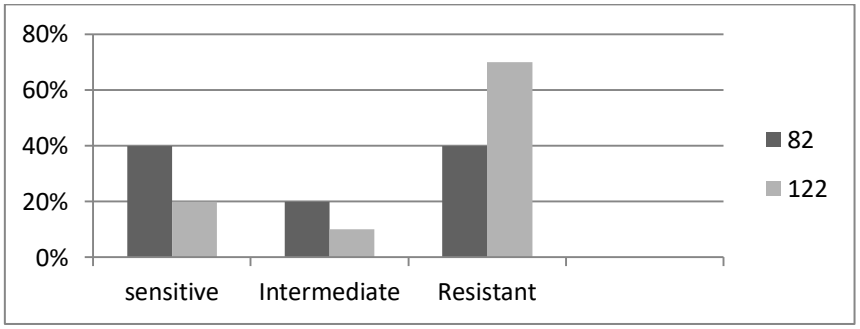

Fig5: The susceptibility test for the most potent isolates toward antibiotic.

The result concluded from this test indicated that the most effective antibiotic that should be used toward the infection caused by *S. aureus* I 82 and *S. warneri* O 122 strains is vancomycin (30 µg). But it's must be known that for many years later and with the abuse of traditional antibacterial agents addition to the decrease of the development of new antibacterial agents, combined with increasing numbers of *S. aureus* strains have become Methicillin Resistant *Staphylococcus aureus* (MSRA) strains, which are spread in communities, leading to dramatic changes in epidemiology and disease incidence during over the world. Consequently, the investigation of new therapeutic agents to avoid the multidrug resistance of *Staphylococcus* spp. was recommended particularly by using some natural compounds upon the level of gene regulation to control in such pathogens.

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التوصيف الأولى والتعريف للبكتريا المسؤله عن انحلال الدم ولموجبه لصبغة جرام

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من أكثر الكائنات الممرضه أهمية والتى تهدد صحة الانسان على مستوى العالم هى المكورات العنقوديه ، لذلك يعتبر التوصيف الاولى والتعريف لمثل هذا الكائن أداه مفيده وقويه للسيطره على الكثير من المخاطرالتى تنتج عن هذا الكائن ، وبالتالى تم عزل 126 سلاله من واقع 126 عينه تتضمن مسحات من: الدم والبول والخراج والسائل المنوى والصديد واللعاب والأذن والبصاق والمهبل من مرضى مستشفى طنطا الجامعى ومن عياداتها الخارجيه ، وكانت هذه العزلات على النحو التالى : 107 عزله كمكورات عنقويه و15 عزله كمكورات عقديه و4 عزلات كمكورات معويه وكن هذا التعريف المبدئى مستندا على أساس توصيف المستعمره : كصبغة جرام وانتاج الصبغات وانحلال الدم ونشاط انزيم الكتاليز واختبار تجلط الدم وتخمير سكر المانيتول وكان ناتج اختبار تجلط الدم كالتالى 30.95% من العزلات لديهم القدره على عمل تكسير كامل لكرات الدم الحمراء و30.95% لديهم القدره على عمل تكسير جزئى لكرات الدم الحمراء بينما كان 38.09% لا يمتلكون القدره على تكسير كرات الدم الحمراء . وتم اختيار سلالات المكورات العنقويه والتى كان لديها القدره على عمل تكسير كلى لكرات الدم الحمراء لدراسة بعض عومل الضراوة بهاعلى اساس اختبار تجلط الدم والذى نتج عنه أن 17.24% من سلاسلات المكورات العنقوديه ذات القدره على عمل تكسير كلى لكرات الدم الحمراء تستطيع عمل تجلط للدم بينما 82.76% ليس لديهم القدره على عمل تجلط للدم وتم دراسة حساسية هذه الكائنات لبعض أشكل المضادات الحيويه وسيتم عمل دراسات مستقبليه للبحث عن تأثير بعض المركبات الطبيعيه على كبح عمل الجينات المسؤلة عن انحلال الدم فى هذة البكتريا.

الكلمات المفتاحية: انحلال الدم ـ بكتريا موجبة الجرام ـ المكورات العنقودية ـ إنتاج انزيم تجلط الدم