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# Determination of Some Virulence Factors in *Staphylococcus* spp. Isolated from Clinical Samples of Different Egyptian Patients

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**Abstract:** *Staphylococcus* spp. is one of the most important pathogens that cause nosocomial infections. This study aimed to isolate and identify *Staphylococcus* spp. from different human clinical samples in Egypt hospitals andstudying some virulence factors of these isolates. Determination of virulence factor is an important in recognition of bacterial invading toolswhich cause pathogenesis, which may serve as new targets in drug development. A total of 50 strains of *Staphylococcus* spp. were isolated from 200 clinical specimens from wound, abscess, sputum, diabetic foot, urine, blood and other sources coming from patients of three Egyptian hospitals. 54% of the isolates were coagulase positive *Staphylococcus* spp. (CoPS) and 46% coagulase negative *Staphylococcus* spp. (CoNS). among (CoPS) strains, 11.1% has the ability to make alpha hemolysis on blood agar and 48.1% has the ability to make beta hemolysis and 40.7% have no ability to make hemolysis. 92.2% of coagulase positive strains have the ability to produce biofilm with 20% strong degree and 7.4% of it has not while 46% of the coagulase negative strains have the ability to produce biofilm with 25% strong degree while 21.7% cannot produce it. Coagulase negative strains have no ability to produce staphyloxanthin pigment, while 22% of coagulase positive strains have the ability to produce it with different degrees and 56% cannot. 22.2% of coagulase positive strains exhibits resistance to oxacillin antibiotic while 22.2% intermediate and 55.5% sensitive, on the other hand, Vancomycin resistant strains were 7.4% of total CoPS while 92.3% sensitive.

**Key words:** *Staphylococcus* spp. · Virulence factors · Coagulase activity · Biofilm

## INTRODUCTION

*Staphylococcus* spp. is one of the important pathogens of humans; these bacteria are widespread in nature and can be recovered from environment or as commensally inhabitants of the skin, mucous membranes and other body sites in humans and animals [1, 2]. It causes various diseases, food poisoning, pneumonia and toxic shock syndrome [3, 4]. *Staphylococcus aureus* is an important pathogen that causes different disorders. These bacteria has a tools with which these bacteria spread to the deeper soft tissues or invade the bloodstream causes severe clinical complications [5]. In addition*, S.aureus*, especially methicillin-resistant *S. aureus* (MRSA), often causes serious problems via nosocomial infection in hospitals [6, 7]. Furthermore, community-acquired MRSA has recently emerged and has been reported to cause serious infectious diseases, sepsis and pneumonia [8, 9]. *S aureus* can secrete several exotoxins, such as hemolysin, enterotoxins, coagulase, toxic shock syndrome toxin-1(TSST-1) and protein A, which are associated with specific diseases [10]. The clinical importance of *S. aureus* is attributed to notable virulence factors, surface proteins, toxins and enzymes, biofilm formation as well as the rapid development of drug resistance [11]. *S. aureus* strains are also have the ability to produce golden carotenoid pigment, staphyloxanthin that acts as a bacterial antioxidant which protects the pathogen from the host's immune system in the form of reactive oxygen species [12, 13]. The aim of this study was to study the occurrence of some virulence factors produced by *Staphylococcus* strains isolated from patients in three Egyptian hospitals.

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## MATERIALS AND METHODS

**Materials:**
**Microorganisms:**

- American type culture collection strain ATCC 29213.
- Clinical isolates:
- (Code A): 33 *Staphylococcus* spp. strains from Arab Contractors hospital.
- (Cod B):11 *Staphylococcus* spp. strains from Banha teaching hospital.
- (Code C): 6 *Staphylococcus* spp. strains from Banha university hospital.

### Media

**Blood Agar Base Medium (Difco) [14]:** Blood Agar is a general purpose enriched medium often used to grow fastidious organisms and to differentiate bacteria based on their hemolytic properties [15]. Also used for maximum recovery of fastidious microorganisms. Blood Agar Base media are specified in standard method procedures for food testing [16]. It consists of 10g/l Meat extract, 10 g/l Peptone, 5 g/l Sodium chloride and 15 g/l Agar. pH adjusted to 7.3± 0.2 before sterilization, after sterilization, cool to 45-50?C, add 5% v/v sterile defibrinated sheep blood to sterile media then mix vigorously.

**Trypticase Soy Agar (Oxoid) [16, 17]:** This medium is used for the cultivation of a wide variety of microorganisms and commonly used as a maintenance medium for culture collections and testing bacterial contaminants in cosmetics [18]. TSA mainly used to determine hemolytic reactions of bacteria [37]. It consists of 15g/l Tryptone (pancreatic), 5g/l Soybean meal, 5g/l Sodium chloride and 15 g/l Agar. PH adjusted to 7.4 ± 2.0 before sterilization.

**Manitol Salt Agar (Difco) [19]:** Mannitol Salt Agar is highly selective and specimens from heavily contaminated sources may be streaked onto this medium without danger of overgrowth [38]. Mannitol Salt Agar is recommended for isolating pathogenic staphylococci from clinical specimens, cosmetics and microbial limit tests [21, 20, 39]. This medium composed of5g/l Enzymatic digest of casein, 5g/l Enzymatic digest of animal tissue, 1g/l Beef extract, 10g/l D- manitol, 75g/l Sodium chloride, 0.025g/l Phenol red and 15g/l Agar. pH adjusted to 7.4 ± 2.0 before sterilization.

### Methods

**Samples Collection:** Specimens were collected from blood, abscess, pus, sputum, urine, wound swabs and cerebral spinal fluid from patients of three Egyptian hospitals; during 2012.

**Identification of *S. aureus*:** Standard microbiological methods for the identification of *Staphylococci* strains were applied. All specimens were inoculated onto manitol salt agar and incubated at 37°C. After incubation, suspect colonies were examined by Gram staining. The colonies with morphologies compatible with *Staphylococcus* spp. were transferred to Tryptic Soy Broth (TSB) (Oxoid) and Tryptic Soy Agar (TSA) (Oxoid). After growth, *staphylococci* were identified on the basis of colony characteristics, Gram staining, pigment production, hemolysis and the following biochemical reactions, Catalase activity, coagulase test (human plasma) and manitol fermentation, finally a full biochemical tests by BIOMERIEUX VITEK2 SYSTEM [20, 22, 40, 41].

### Determination of Some Virulence Factors

**Coagulase Test:** Coagulase test is based on the ability of *S. aureus* to produce a protein product called coagulase. The test is usually carried out to differentiate the pathogenic *S. aureus* from other strains or species of staphylococci. There are two types of coagulase; Bound coagulase (clumping factor) which converts fibrinogen directly to fibrin without requiring a coagulase reacting factor this type can be detected by the rapid slide Coagulase (SC) technique [23]. This test was performed on a clean slide using a sterile dropper; place a small drop of water or saline on the appropriate end of the slide as a control then place a small drop of human plasma on the opposite end of the slide, with a sterilized loop or applicator stick, collect cells from one colony and emulsify the cells in the water (or saline) and then in the drop of plasma, watch for clumping within 10 seconds of adding the bacterial cells to the plasma, The control drop, saline or water, should show no clumping of bacterial cells. The clumping will become more visible if the slide is rocked gently in a figure 8 motion. The second type of coagulase is free coagulase which converts fibrinogen to fibrin by activating a coagulase reacting factor present in plasma which can be detected by the clumping of bacterial cells in the tube coagulase (TC) technique. Free coagulase activity was determined by the method described by Quinn *et al*. [2], several colonies of each organism were

mixed with 0.5 ml of citrated human plasma in a sterile test tube. The tube was incubated at 37°C and examined after 4 and 24 h. Clot formation at either reading was recorded as positive.

**Hemolysin Production:** Alpha-hemolysin was evaluated on TSA supplemented with 5% washed human erythrocytes. The plates were incubated for 24 h at 37°C, when positive samples showed a wide zone of complete hemolysis with blurred edges. Beta-hemolysin was evaluated by plating strains on 5% sheep blood TSA. The plates were incubated at 37°C for 24 h and then overnight at 4°C, when positive strains showed a wide zone of incomplete hemolysis with sharp edges. Non-hemolysis on 5% sheep blood TSA was evaluated as gamma hemolysis [1, 24]. A quantitative hemolysin assay method was adapted from the previous method [25]. The lysis efficacy of human red blood cells was measured with whole cultures of *S. aureus*. In brief, *S. aureus* cells were diluted at 1:100 with an overnight culture in TSB 37°C for 16 h with shaking at 250 rpm. The cell cultures (50 µl including cells and culture supernatant) were added into diluted human red blood cells that had previously been separated by centrifugation at 900xg for 5 min, washed with PBS buffer three times and diluted at 3% of red blood cells in PBS buffer. For hemolytic activity, the mixture was incubated at 37°C for 1 h with 250 rpm shaking. The supernatant was collected by centrifugation at 16,600xg for 10 min and the optical density was measured at 543 nm.

**Biofilm Formation:** Quantitative determination was carried out by the Micro plate method (MP) proposed by Pfaller *et al*. [26] using tissue culture plates of 96 flat bottomed wells. Each well was filled with 0.2 ml of $10^5$ CFU/ml of a bacterial suspension in TSB. After 24h incubation in aerobic condition at 37°C, the contents were aspirated and plates were washed twice with phosphate buffered saline (PBS, pH: 7.2). The wells were stained with 0.1% crystal violet for 2 min. The plates were read in an enzyme-linked immunosorbent assay (ELISA) reader (BioTecan, ELx808) to 492 nm. Sterile TSB was used as a negative control. All the experiments were repeated at least twice and the values of optical density (OD) were then averaged. A three grade scale was used to evaluate the strains slime producing ability by comparing with OD of negative control or cut off (ODc): no biofilm producer or (-):= ODc; (Weak): = 2x ODc; (Moderate): 2x ODc < ~ = 4x ODc; (Strong): > 4x ODc.

**Staphyloxanthin Assay:** The bright golden coloration of this virulence factor facilitates the virulence screening by the simple observation of color [27]. Also, a quantitative carotenoid assay method was adapted from the previous method [28]. In brief, cells were re-inoculated at 1:100 dilution in TSB medium and incubated for 16 h at 37°C. Cells (1 mL) were then collected by centrifugation at 16,600xg for 1 min and washed with 1 ml of phosphate-buffered saline (PBS). At this point, cell pellets were photographed to compare the staphyloxanthin production. For the extraction of carotenoid pigments, the cell pellets were resuspended in 0.2 mL of methanol by vortexing and this mixture was heated at 55°C for 3 min. Pigment extraction was separated from cell debris by centrifugation at 16,600xg for 10 min. This pigment extraction step was repeated 3 times and the optical densities of collected extractions were measured at 465 nm using a spectrophotometer. Each data point was averaged from at least three independent cultures`.

**Oxacillin and Vancomycin Resistance Assay:** The standardized Kirby-Bauer disc-diffusion method was performed on Mueller-Hinton agar using antibiotics oxacillin (1 mg); Vancomycin (30 mg); for testing susceptibility of *S aureus* to either oxacillin as a derivative of methicillin antibiotic for differentiating MRSA strains from the clinical isolates, or Vancomycin for differentiating VRSA strains [29, 30]. In brief, the Trypticase soy agar (TSA) was poured into sterile petri plates and was allowed to solidify. A suspension equivalent to 0.5 McFarland was prepared from each isolate. A swab was dipped and streaked on the surface of a TSA plates. Standard antibiotic discs were introduced on the upper layer of the seeded agar plate. The plates were incubated at 37°C for 18-24h. The experiment was carried out three times and the mean values are presented. The antimicrobial activity was evaluated by measuring the diameter of zone of inhibition in mm.

## RESULTS

**Isolation and Identification of Isolates:** Origin of isolates and species distribution and primary morphological and biochemical identification tests of the *Staphylococcus* spp. is given in Table (1). From a total of 200 clinical samples, 50 (25%) *Staphylococcus* strains were isolated. These isolates were then differentiated into 27 isolates of coagulase positive *Staphylococcus* spp. (CoPS) and 23 isolates coagulase negative *Staphylococcus* spp. (CoNS).

Table 1: Isolation and primary identification of Staphylococcus spp. strains from the collected samples related to their sources

| Sample code | Sample source | Manitol salt agar (MSA) | Gram stain | Catalase test | Coagulase test | |
|---|---|---|---|---|---|---|
| | | | | | Tube | Slide |
| A1 | Blood | + R to Y* | +ve cocci | + | + | + |
| A4 | Wound | + R to R | +ve cocci | + | - | - |
| A7 | Wound | + R to Y | +ve cocci | + | + | + |
| A11 | Sputum | + R to R | +ve cocci | + | - | - |
| A15 | Urine | + R to R | +ve cocci | + | - | - |
| A16 | Diabetic foot | + R to Y | +ve cocci | + | + | + |
| A18 | Blood | + R to Y | +ve cocci | + | - | + |
| A22 | Wound | + R to R | +ve cocci | + | - | - |
| A25 | Ear-swab | + R to R | +ve cocci | + | - | - |
| A27 | Wound | + R to Y | +ve cocci | + | + | + |
| A28 | Sputum | + R to R | +ve cocci | + | - | - |
| A29 | Urine | + R to R | +ve cocci | + | - | - |
| A34 | Wound | + R to R | +ve cocci | + | - | - |
| A39 | Anal abscess | + R to Y | +ve cocci | + | + | + |
| A44 | Pus | + R to R | +ve cocci | + | - | - |
| A46 | Urine | + R to R | +ve cocci | + | - | - |
| A47 | Sputum | + R to R | +ve cocci | + | - | - |
| A55 | Wound | + R to R | +ve cocci | + | - | - |
| A59 | Sputum | + R to R | +ve cocci | + | - | - |
| A63 | Wound | + R to Y | +ve cocci | + | - | + |
| A68 | Nasal swap | + R to Y | +ve cocci | + | - | + |
| A71 | Abscess | + R to Y | +ve cocci | + | + | + |
| A73 | Endotracheal tube | + R to Y | +ve cocci | + | + | + |
| A77 | Wound | + R to R | +ve cocci | + | - | - |
| A80 | Sputum | + R to R | +ve cocci | + | - | - |
| A81 | wound | + R to Y | +ve cocci | + | + | + |
| A86 | Ascetic fluid | + R to Y | +ve cocci | + | + | + |
| A89 | Wound | + R to Y | +ve cocci | + | + | + |
| A91 | Wound | + R to Y | +ve cocci | + | + | + |
| A93 | Sputum | + R to Y | +ve cocci | + | weak | + |
| A95 | Blood | + R to Y | +ve cocci | + | + | + |
| A97 | Wound | + R to R | +ve cocci | + | - | - |
| A98 | Abscess | + R to Y | +ve cocci | + | + | + |
| B3 | Wound | + R to Y | +ve cocci | + | + | + |
| B7 | Blood | + R to R | +ve cocci | + | - | - |
| B10 | Abscess | + R to Y | +ve cocci | + | + | + |
| B11 | Sputum | + R to Y | +ve cocci | + | weak | + |
| B15 | Blood | + R to Y | +ve cocci | + | + | + |
| B19 | Wound | + R to Y | +ve cocci | + | + | + |
| B25 | casther | + R to Y | +ve cocci | + | + | + |
| B26 | Abscess | + R to R | +ve cocci | + | - | - |
| B32 | CSF | + R to R | +ve cocci | + | - | - |
| B33 | Urine | + R to R | +ve cocci | + | - | - |
| B36 | Urine | + R to R | +ve cocci | + | - | - |
| C1 | Wound | + R to Y | +ve cocci | + | - | + |
| C4 | Sputum | + R to Y | +ve cocci | + | + | + |
| C6 | Abscess | + R to Y | +ve cocci | + | + | + |
| C10 | Wound | + R to R | +ve cocci | + | - | - |
| C15 | Blood | + R to Y | +ve cocci | + | + | + |
| C20 | Anal abscess | + R to Y | +ve cocci | + | + | + |

*R to Y: red color of MSA converted to yellow color after incubation, (R to R) red color of MSA not converted after incubation, (+ve) positive, (-ve) negative

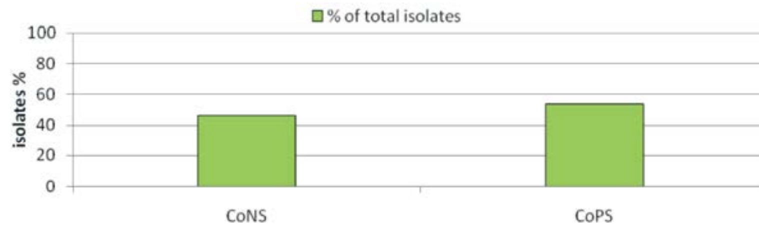


Chart 1: Percentage of coagulase positive and coagulase negative between *Staphylococcus* spp. Isolated from different source

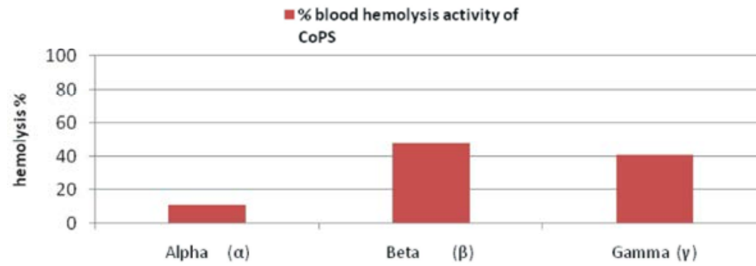


Chart 2: Percentage of blood hemolysis types between coagulase positive *Staphylococcus* spp. isolates

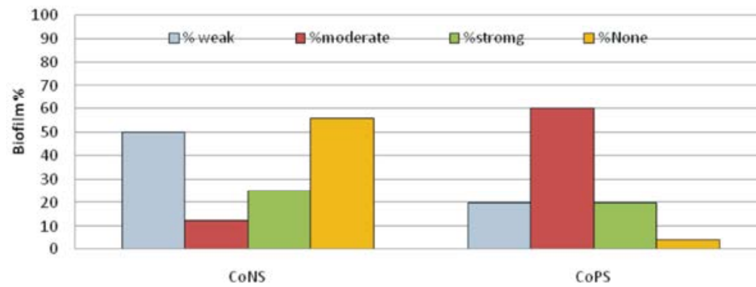


Chart 3: Percentage of biofilm formation between coagulase positive and coagulase negative *Staphylococcus* spp. isolates

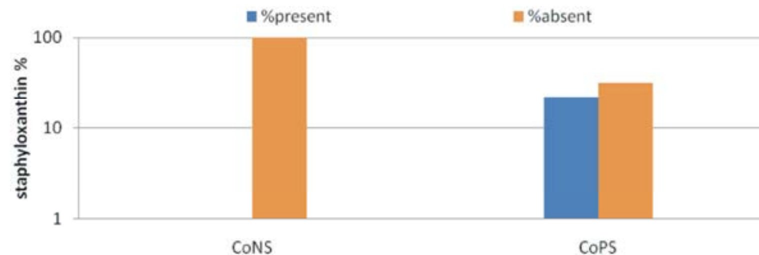


Chart 4: Percentage of staphyloxanthin production between coagulase positive and coagulase negative *Staphylococcus* spp. Isolates

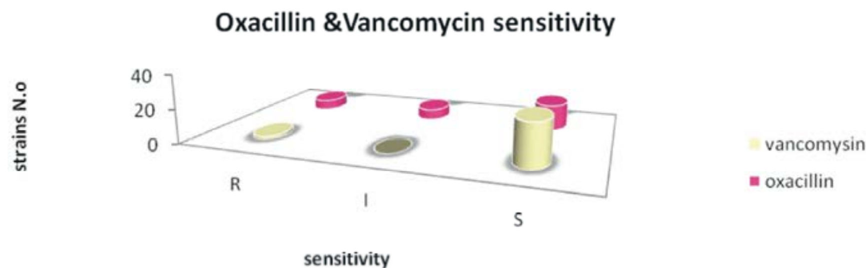


Chart 5: Oxacillin and Vancomycin sensitivity between coagulase positive *Staphylococcus* spp. isolates S: sensitive, I: intermediate and R: resistant

Table 2: Coagulase production test for all *Staphylococcus* spp. Isolates

| Coagulase | 50 strains of *Staphylococcus* spp. | |
|---|---|---|
| | Positive (%) | Negative (%) |
| Bound (SC)* | 27 (54%) | 23 (46%) |
| Free (TC)* | 22 (44%) | 28 (56%) |

*SC: slide coagulase TC: tube coagulase

Table 3: Production ofdifferent types of hemolysis by coagulase positive Staphylococcus strains

| Hemolysis | 27strains of CoPS |
|---|---|
| | Positive (%) |
| Alpha (α) | 3 (11.1%) |
| Beta (β) | 13 (48.1%) |
| Gamma (γ) | 11 (40.7%) |

Table 4: Hemolysis degree of 13 strains producing β hemolysis by quantitative method

| Strain code | *O.D 546nm | Relative hemolysis % |
|---|---|---|
| ATCC 29213 (C +ve)* | 1.014 | 100 |
| A7 (C –ve)* | 0.025 | 0 |
| A16 | 0.321 | 31.6 |
| A27 | 0.531 | 52.3 |
| A39 | 0.24 | 23.6 |
| A68 | 0.55 | 54.2 |
| A81 | 0.853 | 84.1 |
| A91 | 0.947 | 93.3 |
| A95 | 0.582 | 57.3 |
| A98 | 0.084 | 8.2 |
| B3 | 0.226 | 22.2 |
| B11 | 0.325 | 32.1 |
| B15 | 0.911 | 89.8 |
| C15 | 0.996 | 98.2 |
| C20 | 1.045 | 103 |

*OD: optical density * C-ve: control negative, *C+ve: control positive.

## Determination of Some Virulence Factors

**Coagulase Enzyme Production:** Twenty three isolates of *Staphylococcus* species have not the ability to produce both free and bound coagulase enzyme and 27 isolates have the ability to produce coagulase with different degrees as shown in Table (2).

**Hemolysin Production:** Between coagulase positive *Staphylococcus* strains which have high potential to identified as *Staphylococcus aureus* there are some strains have the ability to make alpha, beta and gamma hemolysis and detected visually on blood agar plates, recorded in Table (3). And the positive beta hemolysis or complete hemolysis which can produce an important virulence factor α hemolysin protein with different degrees detected quantitatively by spectrophotometer by using *S. aureus* ATCC 29213as positive control, clinical isolate *S. aureus* C20 exhibit the most áhemolysin producing strain. Results recorded in Table (4).

**Staphyloxanthin Production:** Qualitative determination of staphyloxanthin pigment of *Staphylococcus* clinical isolates showed in Table (6), among coagulase positive isolate A98 identified as *S. aureus* and exhibit the most staphyloxanthin producing strain according to control positive *S. aureus* ATCC 29213, quantitative detection by colorimetric method showed in Table (7).

**Biofilm Formation:** Qualitative and quantitative detection of biofilm formation between fifty clinical isolates of *Staphylococcus* spp. CoNS and CoPS recorded in Table (5).

**Oxacillin and Vancomycin Resistance:** Between the staphylococcus coagulase positive isolates A89 identified as *S. aureus* and exhibit resistance to both Oxacillin and Vancomycin antibiotics as shown in Table (8).

Table 5: Biofilm formation test for all *Staphylococcus* spp. Isolates illustrates strength degree

| Biofilm Formation | 50 strains of *Staphylococcus* spp. | | | | | |
|---|---|---|---|---|---|---|
| | 23*CoNS (%) | | | 27*CoPS (%) | | |
| Present | 8 (46%) | | | 25 (92.5%) | | |
| Strength | Weak | Moderate | Strong | Weak | Moderate | Strong |
| | 5(50%) | 1(12.5%) | 2(25%) | 5(20%) | 15(60%) | 5(20%) |
| Absent | 15 (21.7%) | | | 2 (7.4%) | | |

*CoNS: coagulase negative *Staphylococcus* spp., *CoPS: coagulase positive *Staphylococcus* spp.

Table 6: Qualitative detection of staphyloxanthin pigment production for all *Staphylococcus* spp. Isolates

| | 50 strains of *Staphylococcus* spp. | |
|---|---|---|
| Staphyloxanthin pigment | 27*CoPS (%) | 23*CoNS (%) |
| Present | 11(22%) | - |
| Absent | 16 (56%) | 23 (100%) |
| Total | 27(54%) | 23(46%) |

*CoNS: coagulase negative *Staphylococcus* spp., *CoPS: coagulase positive *Staphylococcus* spp.

Table 7: Staphyloxanthinproduction degree of 11 strains by quantitative colorimetric method

| Sample code | Staphyloxanthin *O.D | Percentage(%) |
|---|---|---|
| ATCC 29213(C +ve) | 0.562 | 100 |
| A25 | 0.087 | 15.4 |
| A27 | 0.125 | 22.2 |
| A71 | 0.539 | 95.9 |
| A73 | 0.111 | 19.7 |
| A98 | 0.551 | 98 |
| B33 | 0.123 | 21.8 |
| B25 | 0.506 | 90.0 |
| C1 | 0.421 | 74.9 |
| C6 | 0.306 | 54.4 |
| C10 | 0.098 | 17.4 |
| C21 | 0.213 | 37.9 |

*OD: optical density

Table 8: Oxacillin and vancomycin susceptibility test for 27 coagulase positive *Staphylococcus* spp. Isolates

| Sample code | Oxacillin | Vancomycin |
|---|---|---|
| A1 | 11mm (I)* | 15 mm (S) |
| A7 | 15 mm (S)* | 18 mm (S) |
| A16 | 0 mm (R)* | 5 mm (R) |
| A18 | 17 mm (S) | 19 mm (S) |
| A27 | 11 mm (I) | 16 mm (S) |
| A39 | 13 mm (S) | 15 mm (S) |
| A63 | 14 mm (S) | 16 mm (S) |
| A68 | 14 mm (S) | 15 mm (S) |
| A71 | 12 mm (I) | 15 mm (S) |
| A73 | 15 mm (S) | 16 mm (S) |
| A81 | 15 mm (S) | 18 mm (S) |
| A86 | 15 mm (S) | 17 mm (S) |
| A89 | 0 mm (R) | 0 mm (R) |
| A91 | 14 mm (S) | 18 mm (S) |
| A93 | 11 mm (I) | 17 mm (S) |
| A95 | 0 mm (R) | 16 mm (S) |
| A98 | 14 mm(S) | 20 mm (S) |
| B3 | 15 mm (S) | 21 mm (S) |
| B10 | 0mm (R) | 22 mm (S) |
| B11 | 11 mm (I) | 16 mm (S) |
| B15 | 0 mm (R) | 18 mm (S) |
| B19 | 12 mm (I) | 16 mm (S) |
| B25 | 15 mm (S) | 17 mm (S) |
| C1 | 14 mm (S) | 15 mm (S) |
| C4 | 0 mm (R) | 17 mm (S) |
| C15 | 20 mm (S) | 18 mm (S) |
| C20 | 16 mm (S) | 24 mm (S) |
| ATCC 29213 | 18 mm (S) | 21 mm (S) |

*S: sensitive, *I: intermediate, *R: resistance.

## DISCUSSION

In this study, the determination of Coagulase activity, hemolysis, biofilm formation, staphyloxanthin production and drug resistance of the *Staphylococcus* spp. clinical isolates was aimed. These virulence factors were considered as bacterial tools for cause pathogenicity and could be detected easily in laboratories [1, 31, 32]. Coagulase enzyme production is usually usedfor differentiate the pathogenic *S.aureus* from other strains or species of staphylococci [23], in our study there are 25% of total clinical specimens were primarily defined as *Staphylococcus* spp. strains which reflect the importance of this type of microorganism that transmitted readily in hospital community and different medical tools. 54% of the isolates were coagulase positive *Staphylococcus* spp. (CoPS) which represent almost pathogenic *Staphylococci* strains such as *S aureus,* while 46% coagulase negative *Staphylococcus* spp. (CoPS). This considered an opportunistic organism and may infecting some medical tools and produce biofilm.

The most important virulence factor in *Staphylococcus* spp. is hemolysins, which causes host cells damaged by making pores in it. [33], which contribute to pathogenicity in *S aureus*. Alpha hemolysin readily lyses sheep, rabbit erythrocytes or human red blood cells. Another one called beta hemolysin has sphingomyelin-specific phospholipase activity, resulting in partial cell lysis [32, 33]. In the present study, it was determined that among (CoPS) strains there are 11.1% have the ability to make alpha hemolysis on blood agar and 48.1% have the ability to make beta hemolysis and 40.7% have no ability to make hemolysis or make gamma hemolysis which indicate in generally, that beta hemolysis was more in CoPS than alpha and gamma hemolysis; this result might have originated from CoPS strains of which nearly 54% of total isolates consisted of *S. aureus* with beta hemolysis characteristics [33, 34].

Examination for biofilm formation could be a useful indicator for the pathogenicity of staphylococci. Slime production may reflect the microorganism's capacity to adhere to specific host tissues and thereby to produce active colonies [35]. In our study there is 92.2% of coagulase positive strains have the ability to produce biofilm with 20% strong degree, 60% moderate degree and 20% weak degreeand 7.4% of it has no ability to producing biofilm.While 46% of the coagulase negative strains have the ability for biofilm production with 25% strong degree,12.5% moderate and 50% weak degree while 21.7% can't produce it. This result confirmed by the

previous study [36] who found that CoPS is more active for biofilm formation than CoNS, they decided that CoPS were represent 74.7% while CoNS were 36.7%.

Pigment, staphyloxanthin that acts as virulence factor, being a bacterial antioxidant which protects the pathogen from the host's immune system in the form of reactive oxygen species [32]. This character is specific to *S aureus* and it ranged from gray to yellow golden color with different degrees [27, 28]. In this study coagulase negative strains have no ability to produce this pigment, while 22% of coagulase positive strains have the ability to producing it with different degrees and 56% have no ability to producing this pigment. This result confirming the suggestion that staphyloxanthin pigment, facilitate pathogenicity of pathogenic types of *Staphylococcus* species by protecting them from free radical which rises in this study that the most commensal probability coagulase negating *Staphylococcus* strains have no ability to make this pigment while the most pathogenic probability coagulasepositive *Staphylococcus* strains has the ability to producing it with different degrees.

The *S. aureus* antibiotic resistance especially methicillin resistant *Staphylococcus aureus* (MRSA) has special interest in the recent years because high mortality rates of it [8, 11, 28]. In our study 22.2% of coagulase positive strains exhibit resistance to oxacillin antibiotic while 22.2% intermediate and 55.5% oxacillin sensitive, in the other way Vancomycin resistant strains were 7.4% of total CoPS while 92.3% sensitive, which indicate that antibiotic vancomycin could be used as antibiotic treatment for MRSA infection. With a recommendation of investigate an alternative therapeutic agents to avoid the multidrug resistance.

## REFERENCES


1. Koneman, E.W., S.D. Allen, V.R. Dowell and H.M. Sommer, 1988. Diagnostic Microbiology. Chapter 9, 5th ed., J.B. Lippincott Co., Philadelphia, USA, pp: 539-576.
2. Quinn, P.J., M.E. Carter, B.K. Markey and G.E. Cartey, 1994. Clinical Veterinary Microbiology.Section 2. Bacteriology, 8. *Staphylococcus* species. Mosby-Year Book Europe Limited, Lynton House, London, England, pp: 118-126.
3. Foster, T.J., 2004. The *Staphylococcus aureus* "superbug". J. Clin. Invest., 114: 1693-1696.
4. Foster, T.J., 2005. Immune evasion by staphylococci. Nat. Rev. Microbial., 3: 948-958.
5. Weinandy, F., K.L. Baath, V.S. Korotkov, T. Bçttcher, S. Sethi, T. Chakraborty and S.A. Sieber, 2014. A β-Lactone-Based Antivirulence Drug Ameliorates *Staphylococcus aureus* Skin Infections in Mice. Chem. Med. Chem., 9: 710-713.
6. Deurenberg, R.H., *et al*. 2007. The molecular evolution of methicillin resistant *Staphylococcus aureus.*Clin.Microbial. Infect., 13: 222-235.
7. Grundmann, H., M. Aires-de-Sousa, J. Boyce and E. Tiemersma. 2006. Emergence and resurgence of methicillin-resistant Staphylococcus aureus asa public-health threat. Lancet, 368: 874-875.
8. Berman, D.S., W. Eisner and B. Kreiswirth, 1993. Community-acquired Methicillin-resistant *Staphylococcus aureus* infection. N. Engl. J. Med., 329: 1896.
9. Deleo, F.R., M. Otto, B.N. Kreiswirth and H.F. Chambers, 2010. Community- associated methicillin-resistant *Staphylococcus aureus*. Lancet, 375: 1557-1568.
10. Ohlsen, K., Koller K.P. and J. Hacker, 1997. Analysis of expression ofthe alpha-toxin gene (hla) of Staphylococcus aureus by using a chromosomally encoded hla: lacZ gene fusion. Infect Immune, 65: 3606-3614.
11. Arvidson, S. and K. Tegmark, 2001.Regulation of virulence determinants in *Staphylococcus aureus*. Int J. Med. Microbial, 291: 159e70.
12. Clauditz, A., A. Resch, K.P. Wieland, A. Peschel and F. Go¨tz, 2006. Staphyloxanthin plays a role in the fitness of Staphylococcus aureus and its ability to cope with oxidative stress. Infect Immune, 74: 4950-4953.
13. Liu, C.I., G.Y. Liu, Y. Song, F. Yin, M.E. Hensler, W.Y. Jeng, V. Nizet, A.H. Wang and E. Oldfield, 2008. A cholesterol biosynthesis inhibitor blocks *Staphylococcus aureus* virulence. Science, 319: 1391-1394.
14. Greenberg, A.E., L.S. Clesceri and A.D. Eaton, (eds.) 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
15. Brown, J.H., 1919. The use of blood agar for the study of *streptococci*. NY Monograph No. 9. The Rockefeller Institute for Medical Research.
16. Labor and Welfare, 2007. Society of Japanese Pharmacopoeia. Amended Chapters 35.1, 35.2, 7. The Minister of Health.

17. Leavitt, J.M., I.J. Naidorf and P. Shugaevsky, 1955. The undetected anaerobe in endodontics: a sensitive medium for detection of both aerobes and anaerobes. The NY J. Dentist., 25: 377-382.
18. Curry, A.S., G.G. Joyce and G.N. McEwen, Jr., 1993. CTFA Microbiology guidelines.The Cosmetic, Toiletry and FragranceAssociation, Inc. Washington, D.C.
19. Chapman, G.H., 1945. The significance of sodium chloride in studies of staphylococci.J. bacteriol. 50:201.20. Kloos, W.E. and T.L. Bannerman 1995. Staphylococcus and Micrococcus. In: P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover and R.H. Yolken (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
20. Greenberg, A.E., L.S. Clesceri and A.D. Eaton. (eds.) 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
21. Hitchins, A.D., T.T. Tran and J.E. McCarron, 1995. Microbiology methods for cosmetics, p. 23.01-23.12. In Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
22. Holt, J.G., N.R. Kreig, P.H.A. Sneath and S.T. Williams, 2001. *Staphylococcus* spp. In Bergey's Manual of Determinative Bacteriology, 9th ed. Baltimore, Williams & Wilkins. USA. 2001; 527.
23. Brown, D.F.J., D.I. Edwards, P.M. Hawkey, D. Morrison, G.L. Ridgway, K.J. Towner and M.W.D. Wren, 2005. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA). J. Antimicrob. Chemother., 56: 1000-1018.
24. Freer, J.H. and J.P. Arbuthnott, 1982. Toxins of Staphylococcus aureus.Pharmacol. Ther, 19: 55-106.
25. Larzabal, M., E.C. Mercado, D.A. Vilte, H. Salazar-Gonzalez, A. Cataldi and F. Navarro-Garcia, 2010. Designed coiled-coil peptides inhibitthe type three secretion system of enter pathogenic Escherichiacoli. PLoS One 5:e9046.
26. Pfaller, M.A., D. Davenport, M. Bale, M. Barrett, F. Koontz and R.M. Massanari, 1988. Development of the quantitative micro-test forslime production by coagulase negative staphylococci. Eur. J. Clin. Microbiol. Infect. Dis., 7: 30-33.
27. Harborne, J.B. and C.A. Williams, 2000. Advances in flavonoid research since 1992. Photochemistry, 55: 481-504-533.
28. Morikawa, K., A. Maruyama, Y. Inose, M. Higashide, H. Hayashi and T. Ohta, 2001. Overexpression of sigma factor, rB, urgesStaphylococcus aureus to thicken the cell wall and to resistb-lactams. Biochem. Biophys Res. Commun., 288: 385-389.
29. Clinical and Laboratory Standards Institute (CLSI.)2009. Methods for dilution antimicrobial susceptibility testing for bacteria that grow aerobically; approved standard, 8th Ed. CLSI document, Wayne, PA. M07-A8.
30. Mamishi, S., S. Mahmoudi, R. Sadeghi, *et al*., 2011. Genotyping of Staphylococcus aureus strains among healthcare workers and patients in the tertiary referral Children's Medical Hospital in Tehran, Iran. Br. J. Biomed Sci., 69(4): 173e7.
31. Otto, M., 2004. Virulence factors of the coagulase-negative staphylococci. Front. Biosci., 9: 841-863.
32. Lee, J.H., J.H. Park, M.H. Cho and J. Lee, 2012. Flavone Reduces the Production of Virulence Factors, Staphyloxanthin and α-Hemolysin, in *Staphylococcus aureus.* Current Microbiology, 65: 726-732.
33. Dinges, M.M., P.M. Orwin and P.M. Schlievert, 2000. Exotoxins of *Staphylococcus aureus*. Clin. Microbiol. Rev., 13: 16-34.
34. Ajuwape, A.T.P. and E.A. Aregbesola, 2001. Biochemical characterization of staphylococci isolated from rabbits. Israel Vet. Med. Assoc., 56: 7-12.
35. Pfaller, M.A., D. Davenport, M. Bale, M. Barrett, F. Koontz and R.M. Massanari, 1988. Development of the quantitative micro-test forslime production by coagulase negative staphylococci. Eur. J. Clin. Microbiol. Infect. Dis., 7: 30-33.
36. Suhyla, T. and K. Osman, 2006. Determination of some virulence factors in *Staphylococcus* spp. Isolated from various clinical isolates. Turk J. Vet. Anim. Sci., 30: 127-132.
37. Orth, D.S., 1993. Handbook of cosmetic microbiology. Marcel Dekker, Inc., New York, NY.
38. Kloos, W.E. and T.L. Bannerman 1995. Staphylococcus and Micrococcus. In: P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover and R.H. Yolken (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
39. Association of Official Analytical Chemists, (AOAC.) 1995. Bacteriological analytical manual, 8th ed., App. 3.08-3.09. AOAC International, Gaithersburg, MD.

40. Espinola, M.B. and W. Lilenbaum, 1996. Prevalence of bacteria in the conjunctival sac and on the eyelid margin of clinically normal cats. J. Small Anim. Pract., 37: 364-366.

41. Cheesbrough, M., 2006. District laboratory practice in tropical countries. Cambridge: Cambridge University Press.