Study the Factors affecting the Production of Coagulase Enzyme from Clinical Bacteria Isolated

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Abstract

This study included isolation and diagnosis of Coagulase Positive Staphylococci bacteria from clinical samples of (21) samples from the Teaching Hospital for Women and Children in Ramadi and included nasal swabs, Ear swabs, Normel skin swabs, Wound swabs, Urine Swabs and Abscesses.

These isolates were diagnosed according to phenotypic and biochemical tests and vatic. After diagnosing the developing colonies, it was found the Gram-positive bacteria formed a percentage (71.42%) of the clinical samples out of the total isolates that gave growth on the culture media, and the Staphylococci positive for the plasma coagulase enzyme (Coagulase Positive Staphylococci) was found. (71.42%) of the total Staphylococci bacteria obtained during isolation and diagnosis of clinical samples and a type of Staphylococcus positive for coagulase (CPS), *Staphylococcus. aureus*, was diagnosed based on the Slide and Tube Coagulase Test.

The sensitivity of CPS-positive staphylococcus to the antibiotic against the bacteria was tested and it was found that the most effective antibiotic against *S. aureus* bacteria was Azithromycin as well as the antibiotic Gentamycin, as indicated by *S. aureus* has demonstrated resistance to Erythromycin as well as Ciprofloxacin.

The different conditions for the production of the plasma coagulase enzyme (Coagulase) for the bacterial isolate of local number (5) were studied, and it was noticed that there is a great similarity in these conditions except for some differences. The study and it was noticed that the appropriate temperature in the production of the enzyme for the local bacterial isolate number (5) is the temperature (35) C, and it was noticed that the pH that led to the increase in the production of the enzyme is (PH10).After (24hr) incubator , and the results were consistent in terms of good production of the enzyme in the mentioned isolation when using a incubator shaker at a speed (100) .

Key words: *coagulase enzyme production , staphylococcus aureus.*

Introduction

Coagulase is an enzyme that is produced by some types of bacteria . The enzyme clots the plasma component of the blood. The only significant disease-causing bacteria of humans that produces coagulase is *Staphylococcus aureus*. The blood clotting enzyme (Coagulase) produced by *S.aureus* bacteria plays a major role in the blood clotting process [1].

Coagulase is an extracellular protein polypeptide consisting of 690 amino acids of molecular weight KDa (70 - 60) containing heterotrophic amino acids [2] . This enzyme consists of three regions first. It is the N-terminous region that contains the Prothrombin binding site and the second is the Central region and

is highly preserved.

coagulase is a prototype of a group of proteins called ZAAPs Adhesion Protein & Zymogen Activator. It has also been observed that some do not consider the plasma coagulase enzyme as an enzyme. Or it may be called ECMBPs) Extracellular matrix binding protein, which gives the surface proteins of bacteria the ability to adhere to some components (ECM) Extracellular matrix For the host[3].

Staphylococci bacteria are the only ones in their production of this enzyme, so we find that this enzyme has received a lot of attention. Staphylococcus is called Staphylococagulase. In 1932, researchers, Beren and Peters Champan, suggested that the plasma coagulant test is important in diagnosing pathogenic bacteria[4].

Materials and Methods

Clinical samples were obtained for all ages and both sexes for the period from (October 5, 2020 to January 25, 2021), which amounted to (21) samples, from the Teaching Hospital for Women and Children in Ramadi, where they were transferred to the hospital laboratory, Bacteriology Department . The clinical samples included nasal swabs, Ear swabs, Normal skin swabs, Wound swabs, Urine Swabs and Abscesses swabs.

S. aureus bacteria were isolated from clinical samples, and grown on mannitol saline agar medium, which is the selection medium for that bacterium. Colonies of bacteria producing clotting enzymes developing on that medium were observed.

The isolates were re-purified by sub-culturing them with several grafts on the medium of the saline mannitol agar, as the developing colonies were taken on the medium of the mannitol agar and were implanted by a planning method with a sterile flame loop vector and the dishes were incubated at 37 ° C for a period of (24- 48 hours) to obtain Pure single colonies.

Microscopic Examination

Microscopy was performed to find out the response of the bacterial isolation to the Gram stain. Part of a growing colony was taken in the middle of Mannitol Salt Agar medium by means of a loop, then a bacterial smear was made from it on a clean glass slide and stained with Gram stain. Then they were examined with a light microscope using the oil lens, and the shape and color of the bacterial cells were observed.

Biochemical Test

Coagulase Test

Tube Coagulase Test:

Free Coagulase was investigated using a tube test, where (0.8) ml of blood plasma was added to (0.2) ml of Brain heart infusion broth medium and inoculated with bacterial isolates growing at an age of (18-24) hour. In small tubes and incubated at a temperature of (37) C for a period of (4) hour , during which the occurrence of coagulation, which indicates the positivity of the test, was monitored, while the tubes in which clotting did not appear at room temperature were left until the next day[7].

Slide Coagulase Test

This method was carried out to investigate the clumping factor enzyme-linked to plasma by using a glass slide and placing a drop of blood plasma on it and then adding to it frish colonies of staphylococcus bacteria at an age of (18-24 hr.) developing in the medium of a Brain heart infusion agar and mixed well, where the appearance of clumping within (5-10) seconds is an indication of the positivity of the test. Another glass slide was used and a drop of bacterial suspension was placed on it with the physiological solution, which represents the negative control[8] .

Statistical Analysis

All experiments were designed according to the SAS - Statistical Analysis System[11] in analyzing the data to study the effect of different parameters on the studied traits according to Complete Randomize Desigen (CRD). The significant differences between the averages were compared with the Least Signification Difference (LSD) test. At a probability level (0.05).

Results and Discussion

The results of the isolates shown in Table (1) showed obtaining 15 coagulase-producing bacterial isolates from 21 bacterial isolates. These isolates varied in their ability to produce the coagulase based on the zone of clotting around the colony developing on the medium of the intestine of gut chickens agar . With a pH of 7 at 37 ° C for a 24-hour incubation period.

The colonies with clotting zone diameters appeared on the medium of the chicken gut, which was used as a nitrogen source.

These samples formed a percentage (71.42%), while the number of samples that gave a negative result for laboratory culture was (6) samples formed a percentage (28.57%), and these results are approximately consistent with what he mentioned[12]. It was found that the percentage of clinical samples that gave a positive growth for laboratory culture was (70%).

Table (1) Bacterial Isolates That Produce Plasma Coagulase Enzyme (Coagulase)

T	NO. Isolation	Location	diameter coagulation (cm)
1	1	Teaching Hospital for Women and Children	4.5
2	5	Teaching Hospital for Women and Children	6
3	6	Teaching Hospital for Women and Children	2.5
4	7	Teaching Hospital for Women and Children	3.5
5	14	Teaching Hospital for Women and Children	3
6	20	Teaching Hospital for Women and Children	2.5
7	21	Teaching Hospital for Women and Children	4.5
8	23	Teaching Hospital for Women and Children	3
9	24	Teaching Hospital for Women and Children	5
10	25	Teaching Hospital for Women and Children	5.5
11	29	Teaching Hospital for Women and Children	3
12	44	Teaching Hospital for Women and Children	5
13	50	Teaching Hospital for Women and Children	5
14	51	Teaching Hospital for Women and Children	7
15	60	Teaching Hospital for Women and Children	4

Figure (1) The percentage of clinical samples that gave growth on the culture media.

The bacterial isolates of the genus Staphylococcus were initially identified based on their phenotypic characteristics when grown on the nutrient agar. Opaque, then it turned pale yellow when growing for a longer period, and this corresponds to what was mentioned .

Staphylococci Sensitivity for Antibiotic

In this study, the sensitivity of selected isolates to. (10) Antibiotic By using the standard antibiotic diffusion method, the bacterium *S. aureus* was shown *S. aureus* under study is resistant to some antibiotics, including the antibiotic Erythromycin, reaching (86.66%). This is close to what was mentioned by Devapriya *et. al*. [17], who obtained a percentage of (88%) of the isolates resistant to this antibiotic.

Wozniak [18] indicated that his isolates of *Staphylococcus aureus* showed resistance to the anti-Ciprofloxacin by (80%) and this is close to what we found, as the isolates resistant to this antibody reached about (73.33%).

Also, the highest sensitivity of *S. aureus* bacteria was obtained with the Azithromycin, where the proportion (86.66%) of *S. aureus* isolates was sensitive to this antagonist, which was mentioned by Laub, Krisztina, *et al*.[19]. The bacterial isolates showed high sensitivity to Gentamycin, as the percentage of sensitive isolates reached (66.66%) , This is close to the findings Swarooprani *et. al*.[20], Where about (72.4%) of the isolates were sensitive to this antagonist.

The results of this study showed that *S. aureus* bacteria isolates under study possessed multiple resistance to antibiotics, which made them one of the pathogens occurring in hospitals as well as their possession of many virulence factors represented by the production of toxins, enzymes and other virulence factors[21].

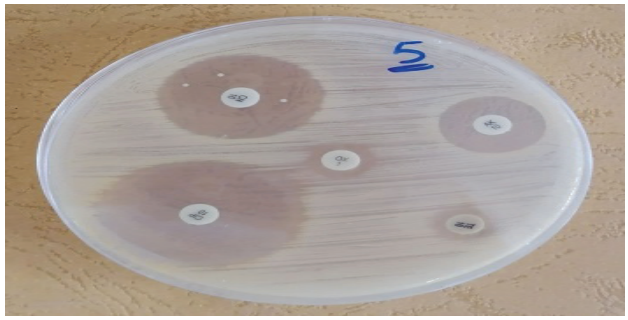

Figure (4) *S.aureus* susceptibility test

Factors affecting of production coagulase :

Effect of pH.

The results of the statistical analysis showed that the pH (10) had a significant difference affecting the productivity of the plasma coagulant enzyme on the rest of the pH numbers of the other isolates (5) of *S. aureus* bacteria.

The pH affects the production of the enzyme through its effect on the properties of the nutrient medium, the solubility of nutrients and their readiness for the organism, and this in turn affects the growth of microorganisms as well as the influence of the enzymes produced by these organisms by the pH of the growing agricultural media.

This result differs with the findings of Wilcox *et al*.[22] , Which explained the use of the pH (7.5) in the production of the enzyme plasma coagulase (Coagulase), and I suggest the reason for this difference to the type, nature and components of the food medium (chicken gut) locally prepared It is considered the first of its kind in such a study.

Table (2) The effect of pH on the production of Coagulase enzyme from Staphylococci isolates.

No.	PH	enzymatic activity. Units / ml
1	5	0.460 ± 0.031 b
2	6	0.425 ± 0.025 b
3	7	0.421 ± 0.022 b
4	8	0.483 ± 0.037 b
5	9	0.643 ± 0.062 b
6	10	1.010 ± 0.077 a
	LSD value	0.328 *
The averages carrying different letters within the same column differ significantly between them. * (P <0.05).		

The effect of temperature.

The effect of temperature on the production of the (Coagulase) from the bacterial isolates under study using different temperatures ranged between (25, 30, 35, 40,45, 50 and 55) C, and it was found that the highest productivity of bacterial isolate (5) for bacteria *S. aureus* at a temperature of (35) C.

The reason for the increase in the productivity of the enzyme at the high temperature may be attributed to the fact that the high temperature affects the speed of enzymatic reactions inside the cell or on some factors that aid the growth of the isolate, such as the decrease in the percentage of dissolved oxygen[23].

The results of the present study agree somewhat with the results of Sturm *et al*.[24], Which demonstrated the use of a temperature of 37 ° C in the production of the enzyme plasma coagulant of *S. aureus*.

Table (3) The effect of temperature on the production of Coagulase enzyme from Staphylococci isolates .

No.	Temperature	enzymatic activity. Units / ml
1	25	0.435 ± 0.025
2	30	0.434 ± 0.022
3	35	0.518 ± 0.037
4	40	0.452 ± 0.032
5	45	0.355 ± 0.026
6	50	0.490 ± 0.029
	LSD value	0.217 NS
NS: Not significant.		

Effect of Inoculum size.

Inoculation of production medium with different sizes of inoculum (0.5, 1, 1.5, 2, 2.5, 3 ml / ml medium) with uniform density 8 x 10^{-5} colonies / ml of *S. aureus* bacteria for isolation (5). To find out its effect on the production of (Coagulase), the results show that the highest production of the enzyme from *S. aureus* bacteria when adding the inoculum volume is 3 ml / 100 ml.

These results are in agreement with what was obtained[25] , that the best inoculum size for producing coagulase from *S. aureus* was when using a vaccine volume of 3 ml / 100 ml.

Table (4) The effect of the inoculum size on the production of Coagulase enzyme from Staphylococci isolates.

No.	The size of the inoculum	enzymatic activity. Units / ml
1	0.5	0.835 ± 0.082
2	1	0.853 ± 0.071
3	1.5	0.851 ± 0.092
4	2	0.923 ± 0.156
5	2.5	0.887 ± 0.087
6	3	0.978 ± 0.079
	LSD value	0.187 NS
NS: Not significant.		

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