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Isolation and Identification of Hyaluronidase Producing *Staphylococcus aureus*

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Abstract:

In this study, a total of one hundred clinical samples were collected for isolation of *Staphylococcus aureus* from different hospitals (Al Yarmouk Teaching Hospital and Hospital Child Central and a number of Al- Harthiya laboratories) in Baghdad.

The diagnostic results showed that 34 out of 100 samples were beloned to gram positive bacteria and 66 were gram negative according to their morphological and cultural characteristics. From these 34 gram positive isolates 25 isolates were confirmed as *Staphylococcus aureus*, 6 *Staphylococcus epidermidis* and 3 *Staphylococcus saprophytic us*.

These *S. aureus* isolates were tested for their ability to produce hyaluronidase enzyme and results showed that all the isolates were hyaluronidase producers; among them, *S. aureus* isolate S2 obtained from urine was the most efficient in hyaluroniase production. Enzyme specific activity of the crude filtrate of this isolate was 96 U/mg proteins.

الخلاصة:

جمعت 100 عينة سريرية وقد أظهرت النتائج أن 34 من 100من العزلات تعود الى ملون الغرام الموجب و 66 منها تعود الى ملون الغرام السالب.

شخصت العزلات وفقاً لخصائصها المزرعية والمظهرية. أخضعت جميع هذه العزلات للاختبارات الكيموحيوية المختلفة وقد وجد أن 25 من 34 تعود الى *Stapylococcus aureus* و 6 تعود الى *Staphylococcus epidermidis* و3 تعود الى *Staphylococcus saprophyticus* وقد اختبرت قابلية العزلات المشخصة في إنتاج انزيم الهالرونيديز، وقد وجد ان *S. aureus* رقم 2 المعزولة من الادار هي الأكفأ في إنتاج الإنزيم حيث وصلت الفعالية الانزيمية الى 96 وحدة/ملغم بروتين.

Introduction:

The hyaluronidases (HYALs) are a group of enzymes that regulate hyaluronic acid (HA) metabolism and consequently remodel the extracellular matrix (ECM**),** these enzymes are produced by: mammals as a component of seminal fluid, plasma, urine and in most tissues of the body [1], bacteria as a virulence factor [2], and venomous animals as a non toxic component of venoms [1]**.**

Hyaluronic acid, a polysaccharide found in the intercellular ground substance of connective tissue, and of certain specialized tissues, such as the umbilical cord and vitreous humor [3]**.**

Hyaluronidase cleaves glycosidic bonds of hyaluronic acid and, to a variable degree, some other acid mucopoly saccharides of the connective tissue. Hyaluronidase has a panoramic use in biotechnology processes and therapy due to its therapeutic, path physiological, physiological and biological importance, also it has been used therapeutically due to their capacity to reduce biological fluid viscosity, increase vascular permeability and render tissues more accessible to certain drugs [4].

The ideal cancer treatment should be able to eradicate systemic tumors at multiple sites in the body and have the specificity to discriminate between neoplastic and non-neoplastic cells, in this regard; antigen-specific cancer immune-therapy represents an attractive approach for treatment [5].

Enzymes as drugs have two important features the first they often bind and act on their target affinity and

specificity, the second they are catalytic and convert multiple target molecules to desired products; these two features make enzymes specific and potent drugs for a wide range of disorders [6].

Tumor-associated HA and hyaluronidase system is known to promote tumor growth and metastasis [7].

Enzyme therapies are becoming more common in disease treatment, the recent research on hyaluronidase enzyme shows that the hyaluronidase enzyme from bacterial isolate, an HA synthesis inhibitor, has antitumor activity in prostate cancer cells, tumor tissues and bladder cancer, also used as myeloid stimulating factor [8].

On the other hand, high cost of developing drug therapies for rare diseases and pharmacoeconomic concerns makes difficult to meet the particular needs of patients with rare diseases that lead to use microbial enzymes because they are generally cheaper to produce; their enzyme content is more predictable and controllable, reliable supplies of raw material of constant composition, but some of them are incompatible with the human body [9].

For a long period of time the hyaluronidases were a group of poorly characterized, neglected enzymes [10].

Hyaluronidase that cause bacterial hyaluronate lyases were reported to be virulence factors that facilitate the spreading of bacteria in host tissues by degradation of HA[11].

The lyses of hyaluronic acid by hyaluronidase enzymes facilitates invasion, and adhesion, and colonization of bacteria in the host's body and spread from the initial site for injury to the rest of the body [12].

Based upon the medical, physiological, biological and commercial importance of hyaluronidase, authors have screened and isolated a newly promising bacterial strain such as *Streptococcus pyogenes, Streptococcus pneumonia, Clostridium welchii, and Staphylococcus aureus* with higher yield followed by its characterization employing detailed taxonomic studies [13].

Materials and Methods:

1- Sample Collection:

Samples included urine, blood, suptum, and swaps from different patient's body (ear, eye, wound, burn, and nose) vagina and abscesses were collected from Al- Harthiya civil laboratories in Baghdad, Al-Yarmouk Teaching Hospital and Hospital Child Center.

2- Isolation of *Staphylococcus aureus*:

For the isolation of S. *aureus* all specimens were inoculated on blood agar media and mannitol salt agar media. Cultural characteristics were studied depending on colonies characteristics of suspected isolate (color, size, shape, edges and high) on the surface of manitol salt agar media and Blood agar media.

3- Identification of the isolates:

Samples processed for microsco-pical examination using Gram stain to examine cell shape, grouping, size and Gram reaction. Then some biochemical test was achieved such as catalase test, oxidase test, coagulase test, DNase test, growth on mannitol salt agar, growth on Blood agar, and urease test [14].

Results and Discussion:

One hundred clinical samples were collected from different hospitals. The samples of wound swab, burn swab, ear swab, urine, vaginal, abscesses and blood were collected from different patient attending these hospitals.

Results showed that (34) out of 100 samples were belong to Gram positive bacteria, and (66) samples showed the growth of gram negative bacteria when cultured on blood agar medium and examined microscopically, also it was found that from 34 samples 25 belong to

S. aureus, 6 belong to *S. epidermidis* and 3 *S. saprophyticus* (figure-1).

It was found that the proportion of isolates these bacteria from various sources of the body (ear, nose and wounds, pharynx, ulcers and lactation and vagina) were 35.71% [15].

Also it was mentioned that the ratio of isolated bacteria was 29.5%, these varying ratios in isolate the bacteria due to the difference in the season of sampling, number and source of isolation[16].

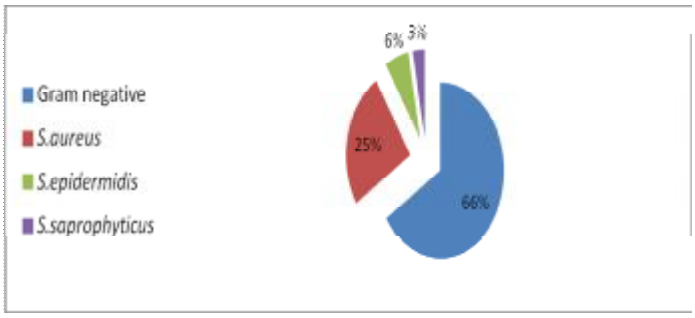

Figure-1: Prevalence of gram positive and gram negative among clinical samples of patient.

Identification of *Staphylococcus* sp.:

From 100 cases of different patients, 7 from 35 urine samples, 4 from11 ear swaps, 6 from 18 burns samples, 2 from 7 wounds, 2 from 5 vagina, 1 from 9 nose, 1 from 5 blood, 1 from 4 eye, 1 from 6 sputum samples these result showed that twenty five of these isolate were cocci arranged grape like clusters, non spore forming, non motile [14].

It could be concluded that these twenty five isolates were belong to *S. aureus* [17].

Table-1: Biochemical tests for identification of *S. aureus* isolates

Test	Result
Catalase	+
Coagulase	+
DNase	+
Mannitol salt agar	+
Blood Agar	+
Urease	+

Qualitative and quantitative detection of hyaluronidase enzyme:

1-Qualitative screening for *Staphylococcus aureus*

In order to test the ability of *S. aureus* isolates to produce hyaluronidase enzyme, the inhibition zones was measured by using brain heart serum albumin medium (that have 1% bovine serum albumin and 400 µg/ml hyaluronic acid) and it was found that *S. aureus* isolates were hyaluronidase enzyme producing with a zone of hydrolysis ranged between 4-18 mm among them the isolate *S. aurus* 2 was the most efficient in hyaluronidase production because it gives the highest diameter of hydrolysis (18mm) on brain heart serum albumin medium (figure-2). Interaction of HA with BSA lead to formation of white complex precipitate with appearance of clear zone around producer isolates [18].

Solid medium is the best method used by many researchers; it was applied

to investigate detection the hyaluronidase by both gram positive and gram negative bacteria. It is easy to implement method and their results are visible [19]. This method used by many researchers, it was applied to investigate detection the hyaluronidase by both gram positive and gram negative bacteria. This method used to detect the activity of hyaluronidase enzyme produced from H4489A phage that infected *Streptococcus pyogenes* bacteria after the transfer of genes which responsible for the production of the hyaluronidase enzyme to the *E.coli* bacteria [20].

The same way for the qualitative detection of the enzyme in *Streptococcus suis* bacteria[21].

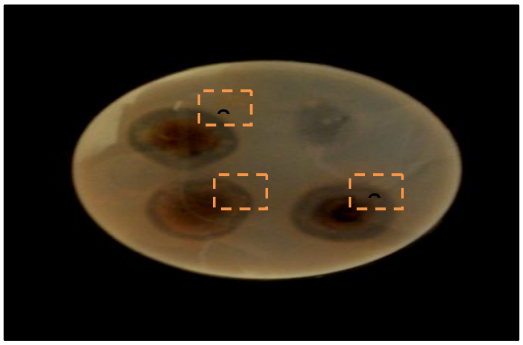

Figure-2: Diameter of hydrolysis zone around *S. aureus* isolates showing hyaluronida se production at37°C after 24hrs.

Table-2: Diameter of clear zone around colonies of *S. aureus* grown on brain heart serum albumin for 24 hours at 37°C.

Symbole	Diameter of clear zone (mm)	Symbole	Diameter of clear zone (mm)
S1	11	S14	5
S2	18	S15	7
S3	11	S16	7
S4	13	S17	5
S5	9	S18	7
S6	10	S19	4
S7	10	S20	8
S8	10	S21	8
S10	9	S22	4
S11	6	S23	7
S12	9	S24	8
S13	6	S25	8

2. Quantitative detection hyaluronidase enzyme:

In order to determining the efficient *S. aureus* in the hyaluronidase production several bacterial isolates was selected depending on the formation of inhibition zone around the producer isolates.

The enzyme activity for each one was determined; figure-3 showed that the specific activity of enzyme from these isolates was ranged between (33-96) U\mg proteins.

From this result shown in the figure we choose the isolate number S2 that was more efficient in the production hyaluronidase.

The differences in the production of hyaluronidase enzyme from different isolates due to the difference in the ratio of expression of genes encoded enzyme[19].

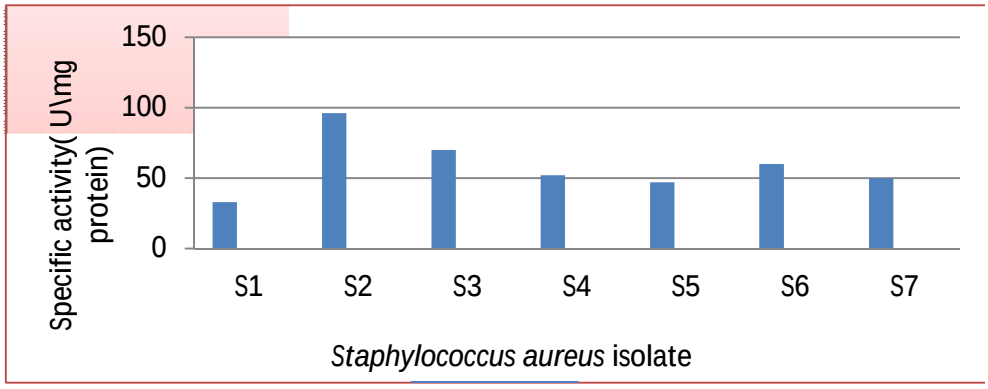

Figure -3: The efficiency of *Staphylococcus aureus* for hyaluronidase production

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