Study the antibiotics sensitivity and beta-lactamase productivity of some Staphylococcus spp. Isolates from different sources of the Al Jamhoree Teaching Hospital in Mosul City

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ABSTRACT

The study includes the isolation of certain types of Gram-positive bacteria Staphylococcus spp. The sample materials (blood, wounds, burns) were collected from both genders of all age groups of inpatients in Al Jamhoree Teaching Hospital in the Mosul during June till end of November 2018.

Staphylococcus aureus was the most common among the isolates with 7 isolates (38.9%) followed by Staphylococcus epidermidis, Staphylococcus simulans with 2 isolates each with 11.1% followed by Staphylococcus chromogenes, Staphylococcus scapilis and Staphylococcus xylosus with one isolate for each one 5.5%. The number of isolates was 9 isolates and 50%, followed by wound samples. The isolates were 6 isolated by 33% and the samples of burns were 3 isolates. 17% were isolated and Staphylococcus aureus was the most dominant species.

The sensitivity of bacterial isolates was studied for 15 antibiotics for different antibiotic groups. The results showed that a difference in the resistance ratio of the isolates to these antagonists, as they were resistant to the Ceftrixone, Cloxacillin 100%. The Ciprofloxacin, Gentamycin, Pipracillin, and Amikacin resistance were reduced. The Ciprofloxacin antagonist was the most affected on the bacterial isolates studied, followed by the Rifampin. The results showed that the beta-lactamase enzyme was not produced by any of the iodic methods by 4 bacterial strains, while the rest of the species varied in the susceptibility of production to the enzyme. The Iodine tube is one of the best methods to detect the production of these enzymes.

The susceptibility of bacterial species to the production of large-spectrum beta-lactase enzymes was also tested using the National Committee for Clinical Laboratory Standards (NCCL) and the double-disc method. Staphylococcus lentus, Staphylococcus capilis, Staphylococcus chromogenes The first NCCL has shown a single isolation of Staphylococcus capilis ability to produce it.

Introduction

Staphylococcus aureus bacteria was first discovered in 1880 by Scottish surgeon Alexander Ogston, which caused. a serious threat to human health. It became one of the worldwide leading causes of hospital infection [1]. In the early 1940s, staphylococcus aureus caused fatal diseases, with an estimated 80% mortality rate [2]. In the mid-1950's, penicillin-resistant gold staphylococci increased to such an extent that penicillin became ineffective in its treatment [3] and
caused an epidemic disease in late 1950 and early 1960 [2]. *Staphylococcus aureus* got more interest because of its increased resistance to many antibiotics, which gave rise to a common health problems worldwide [4]. These bacteria may cause many infectious conditions such as urinary tract and respiratory infection, bacteremia, wound inflammation, and hospital infections. [5]

**Materials and Methods**

Cultures media were used: Nutrient Broth, Blood agar, and Mannitol Salt agar

These medias prepared according to Oxoid and Hemedia companies. The medias were Sterilized with autoclave at 121 °C for 15 minutes. Then kept in the refrigerator at 4 °C until usage.

**Identification and Isolation**

A total of 136 clinical specimens were collected. 56 of which were urine, 44 were swabs from burn, and 36 were swabs from wound. All inpatients were hospitalized in Aljamhoree Teaching Hospital in Mosul city. The first isolates, which included blood agar and mannitol salt agar, were incubated at temperature 37 °C for 18-24 hours. 18 gram positive bacteria identified. Diagnosis of the species and Genus recognized from culture and morphological tests and the microscopic and chemical tests used to isolate and diagnose the bacteria. The results of the diagnosis were supported by the diagnosis of the API Staph. and Vitik Compact system 2.

**Antibiotic Susceptibility Test**

Fifteen antibiotics were used by Bioanalyse. The tests performed according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI), (1986), the standard Kirby & Bauer (1966) standard was used to test antibiotic susceptibility to the antibodies known as Public health (2014) [6]. Several young colonies were transferred to 5 mL container tubes from the center of the nutrient broth and incubated at a temperature of 37 °C for 18-20 hours and a nearby bacterial suspension in tube number (0.5) of standard McFarland tubes, which is equivalent to 8101 cell / cm3, followed by microbial feeding by a sterile cotton scanner taken from suspension A cotton swab and Petri dishes were homogenized on the surface of the dish, left to dry for 10 minutes after being distributed. The tablets were placed at a standard distance equal to the non-interference of the inhibition zones. Added 5-6 tablets /dish followed by incubating the dishes at a temperature of (37) °C for 20-20 hours. Then the inhibition area was measured by millimeter and compared with the standards found in the laboratory constants [7].

**Detection of β-lactamases**

In order to determine the relationship between resistance bacteria and bacterial production of β-Lactamase enzymes, the presence of the enzyme in the bacteria was investigated in the following ways:

1- **Iodometric Method:**

This method is based on the fact that penicillin G is given when the penicillic acid is dissolved, Pencillic acid, which reduces iodine and thus works to shorten the color of the complex starch - iodine Starch-Iodine complex for methods containing starch in the detector components. Two methods were used:

**Iodometric Spot Method:**

2.5 cm of filter paper placed at the bottom of petri dish, at the middle of this paper 0.02g of penicillin G powder placed and few drops of distilled water added to the powder sample of the young colony and mixed with penicillin in the centre of the paper, then left for 10 minutes at room temperature and then add 3-5 drops of Grams' iodine transformer so that the whole paper is included. The result was read within (5) minutes of addition of iodine as the appearance of a white spot in the center of the paper indicates the positive result. [8]

**Tube Method Iodometric**

A number of container tubes were used on 0.1 mL of penicillin G solution per tube. Several colonies were then transferred to the tube and the tubes were left at room temperature for 30 minutes. A drop of Grams iodine solution was added and two drops of starch solution were added Led to the emergence of a dark blue color to the interaction between starch and iodine, and mixed the tube well and left at room temperature, the appearance of white and disappearance Blue in less than 10 minutes is evidence of the production of β-lactamase enzymes [9][10].

2- **Acidimetric Methods**

The basis of this method is to analyze the beta-lactam loop, which generates a carboxyl group that reduces the pH of the medium and can be investigated in test tubes or filter sheets. The study used the tube method as follows:

Preparing the reagent by adding 2 cm 3 of the red phenol solution and add 16.6 cm 3 of the distilled water. Then add 1.2 g of penicillin G, adjust the pH to 8.5 by using 1 NaOH solution. Keep the violet detector at a temperature of -20 °C until use.

To test, 100 µL of reagent was added to each tube. The tubes were fertilized with young germs to produce thick bacterial suspensions and left at room temperature. Within 5 minutes, the yellow color was shown to be evidence of the presence of beta-lactamase enzymes [11].

**Detection of Extended - Spectrum β - Lactamases**

Two methods were used to investigate these enzymes, as follows:

1- **Detection by NCCLS Method**

The National Committee for Clinical Laboratories Standards (NCCLS) has been used to investigate this group of enzymes based on the values of MIC inhibitors for cefotaxime, ceftriaxone, cefazidime, and cefepime. If the MIC values for these antibiotics
are higher than or equal to 2 μg/Cm 3) or cefpodoxim with a value greater than or equal to 8 μg / cm3. The ESBLs are suspected of producing ESBLs and primary and confirmed screening tests are performed to produce these enzymes [12].

**Initial Screening Test:**
An equivalent bacterial suspension in the first tube of 0.5 m of standard McFarland tubes was then subjected to the salinity of the nutritious. Cefotaxim, ceftriaxone and ceftazidime were distributed on the middle surface at equal distances and incubated at 37°C for 16-18 hours. The inhibition area was then compared with the NCCLs for this test. The positive isolates were determined for this test.

**2- Detection By Double Disc Synergy Method**
This method is based on the effect of inhibitory clavolin on ESBLs enzymes. Prepare a microbial suspension of the first tube (0.5) of the standard McFarland tube and then rinse the center of the nut with these bacterial suspensions and leave for 5-15 minutes at room temperature to dry and place the Augmentin tablet at a concentration of 30 μg / tablet. In the dish, ceftazidime tablets were placed at a concentration of 30 μg/tablet and piperacillin at a concentration of 100 μg / tablet (20-30 mm) from the center of the Augmentin. Incubate the dishes at a temperature of (37) m for (24) hours, when a widening occurs in the inhibition zone between the central disc and one or more. Most of the tablets are indicative of the positive result of the production of wide-spectrum beta-lactase enzymes [13] [14].

**Result and Discussion**
The results of the biochemical tests showed the diagnosis of 8 types of cluster bacteria as shown in Table (1) and distributed according to the sources of isolation.

Table 1 showed that isolates were divided by 7 isolates of Staphylococcus aureus with 38.9% and 2 isolates of Staphylococcus sciuri, Staphylococcus lentus, Staphylococcus epidermidis, St. simulans by 11.1% and isolates of Staphylococcus xylosus, Staphylococcus chromanogenes, Staphylococcus capitis by 5.5%.

As for the isolation of Staphylococcus bacteria from the cases of the disease, the study showed that the highest percentage of isolates of bacteria for patients with urinary tract infections with 50% infection followed by burns and surgical injuries. The results were consistent with the study of Alkhafaji [15], which found that the proportion of Staphylococcus isolates was 33% of burn injuries, the current isolation rate was relatively low compared with some studies [16]. Insulation ratio with[17] Differences in isolation rates differ in the number of samples taken, the criteria used for isolation and diagnosis, and the environment and health conditions of patients [18].

| Bacteria                  | Number | Source of isolation | Ratio |
|---------------------------|--------|---------------------|-------|
| Staphylococcus sciuri     | 2      | Urin                | 11.1  |
| Staphylococcus lentus     | 2      | urin wound          | 11.1  |
| Staphylococcus epidermidis| 2      | urin                | 11.1  |
| Staphylococcus simulans   | 2      | urin wound          | 11.1  |
| Staphylococcus xylosus    | 1      | Urin                | 5.5   |
| Staphylococcus chromanogenes | 1  | Wound               | 5.5   |
| Staphylococcus capitis    | 1      | Wound               | 5.5   |
| Staphylococcus aureus     | 7      | Urin2-wound2-burn3  | 38.9  |

Table (1): Determination and percentages of Gram positive bacteria staphylococcus isolated from different sources and their isolation ratios

| Bacteria                  | CRO | AK | OX | F | E | RA | PKL | CFM | CIP | CX | AMC | CTX | CN |
|---------------------------|-----|----|----|---|---|----|-----|-----|-----|----|-----|-----|----|
| St. lentus                | R   | S  | S  | R | R | MS | S   | S   | R   | R  | R   | R   | S  |
| St. aureus                | R   | S  | S  | R | R | S  | R   | S   | R   | R  | R   | R   | S  |
| St. sciuri                | R   | R  | R  | S | S | S  | R   | R   | S   | R  | R   | R   | S  |
| St. simulans              | R   | S  | R  | S | R | S  | R   | S   | R   | R  | R   | R   | S  |
| St. sciuri                | R   | S  | R  | S | S | S  | S   | R   | R   | S  | S   | S   | S  |
| St. capitis               | R   | R  | R  | R | R | R  | R   | R   | R   | R  | R   | R   | S  |
| St. epidermidas           | R   | S  | R  | S | S | S  | R   | S   | R   | R  | R   | R   | S  |
| St. aureus                | R   | S  | S  | R | S | S  | S   | R   | S   | R  | R   | R   | S  |
| St. chromanogenes         | R   | S  | R  | R | R | S  | S   | R   | S   | R  | R   | R   | S  |
| St. aureus                | R   | S  | S  | R | S | S  | S   | R   | S   | R  | R   | R   | S  |
| St. epidermidas           | R   | R  | R  | R | R | MS | MS  | S   | R   | R  | R   | R   | S  |
| St. aureus                | R   | S  | S  | R | S | S  | S   | S   | R   | R  | S   | R   | S  |
| St. aureus                | R   | S  | S  | S | S | S  | S   | R   | S   | R  | R   | R   | S  |
| St. lenteus               | R   | S  | MS | S | R | S  | S   | S   | R   | R  | R   | R   | S  |
| St. simulans              | R   | R  | R  | R | R | R  | R   | R   | R   | R  | R   | R   | S  |
| St. xylosis               | R   | R  | R  | R | R | R  | R   | R   | R   | R  | R   | R   | S  |
| **Total Resistant ratio** | 100%| 33%| 66%| 66%| 66%| 22%| 39% | 89% | 11% | 100%| 95% | 55% | 27%|

| Bacteria                  | CRO | AK | OX | F | E | RA | PKL | CFM | CIP | CX | AMC | CTX | CN |
|---------------------------|-----|----|----|---|---|----|-----|-----|-----|----|-----|-----|----|
| Staphylococcus sciuri     | 2   |    |    |   |   |    |     |     |     |    |     |     |    |
| Staphylococcus lentus     | 2   |    |    |   |   |    |     |     |     |    |     |     |    |
| Staphylococcus epidermidis| 2   |    |    |   |   |    |     |     |     |    |     |     |    |
| Staphylococcus simulans   | 2   |    |    |   |   |    |     |     |     |    |     |     |    |
| Staphylococcus xylosus    | 1   |    |    |   |   |    |     |     |     |    |     |     |    |
| Staphylococcus chromanogenes | 1  |    |    |   |   |    |     |     |     |    |     |     |    |
| Staphylococcus capitis    | 1   |    |    |   |   |    |     |     |     |    |     |     |    |
| Staphylococcus aureus     | 7   |    |    |   |   |    |     |     |     |    |     |     |    |
Ceftrixon, Cloxacillin, Ciprofloxacin, Gentamicin, Amikacin, Rifampin, Erythromycin, Nitrofurantoin, Imipenem, Cefazidim, Cefotaxime, Ceftriaxone, Oxacillin, and Augmentin. The results showed high resistance to Ceftraxone, Ceftriaxone, 100% resistance. The isolates showed high resistance to Augmentin, Cefotaxim, and Ceftriaxone, 95% and 89%, respectively. The results showed sensitivity to Ciprofloxacin, Rifampin, Gentamicin, Amikacin, and Pipercillin. The resistance ratio was 11%, 22%, 27%, 33% and 39% respectively. For Erythromycin, Nitrofurantoin, Oxacillin, (66%) and Cefotaxim (45%). Staphylococcus capitis, Staphylococcus lentus, were more resistant to antibiotics and 92% and 84%, respectively. Staphylococcus aureus bacteria are different. The sensitivity of these bacteria varies according to geographic regions and the health awareness of how antibiotics are used by patients, which increases the resistance of pathogenic bacteria, which in turn increases the health problems. This is consistent with the findings of researchers in different geographical areas, as they recorded high resistance to isolates [19]. Resistance to staphylococcus isolates of beta-lactam antibiotics may be due to their ability to alter target sites or to reduce cell membrane permeability or to produce beta-lactamase enzymes encoded by plasmid-dependent chromosome determinants or may be due to indiscriminate and extensive use of antibiotics. The resistance ratio is a genetic response and makes its difficult to treat the infections caused by these bacteria [20] [21].

Detection of Beta - lactate Enzymes in Isolated Bacterial Species

Beta-lactamase Enzymes were investigated in the isolates of Staphylococcus isolates using three methods: the Iodic tube method, the Iodic spot method and the acidic method. As shown in Table 3, the ratios varied according to the methods used to detect these enzymes. Staphylococcus epidermis showed its ability to produce the enzyme 100% in the iodide method and 50% in the iodine tube method. Staphylococcus simulans showed their ability to produce 100% by using the Iodic spot method. Staphylococcus capitis was produced in all ways except the acidic method.

Table (3) Numbers and percentages of production of beta - lactate enzymes for three - way positive bacteria.

| Bacteria                  | Acidimetric Methods | Iodometric Tube Method | Iodometric Spot Method |
|---------------------------|---------------------|------------------------|------------------------|
| Staphylococcus sciuri     | 0                   | 1                      | 0                      |
| Staphylococcus lentus     | 0                   | 1                      | 1                      |
| Staphylococcus epidermis  | 0                   | 1                      | 2                      |
| Staphylococcus simulans   | 0                   | 0                      | 2                      |
| Staphylococcus xylosus    | 1                   | 0                      | 0                      |
| Staphylococcus chromogenes| 0                   | 1                      | 0                      |
| Staphylococcus capitis    | 0                   | 1                      | 1                      |
| Staphylococcus aureus     | 0                   | 0                      | 3                      |
| Total                     | 5.5%                | 27%                    | 50%                    |

The results of the study were investigated for 5 isolates of total isolation and 28% for the production of high-density beta-lactase enzymes in the double-disc method and isolation Only one of Staphylococcus lentus, Staphylococcus Chromogenes, Staphylococcus capitis and two isolates of Staphylococcus aureus, while one isolating type Staphylococcus capitis showed its ability to produce this enzyme in NCCL method as shown in Table (4).

Table (4) Numbers and percentages of isolates produced for the broad - spectrum beta - lactate enzymes

| Bacteria                  | NCCLS Method | Double Disc Synergy Method |
|---------------------------|--------------|----------------------------|
| Staphylococcus sciuri     | 0            | 0                          |
| Staphylococcus lentus     | 0            | 1                          |
| Staphylococcus epidermis  | 0            | 0                          |
| Staphylococcus simulans   | 0            | 0                          |
| Staphylococcus xylosus    | 0            | 0                          |
| Staphylococcus chromogenes| 0            | 1                          |
| Staphylococcus capitis    | 1            | 1                          |
| Staphylococcus aureus     | 0            | 2                          |
| Total                     | 5.5%         | 27%                        |

The results of the examination of the production of beta-lactase enzymes for Staphylococcus isolates in the three methods shown in Table 3 that (9) isolates out of 18 isolates and 50% showed their ability to produce the enzyme. These results were higher than those of [22]Enzyme 30%. The introduction of the beta-lactase enzyme test as a routine test of the disease isolates gives an early evidence for the efficacy of anti-treatment, which saves effort and
time and avoids the use of antibiotics sensitive to these enzymes in treatment. All isolates were tested for the wide-spectrum beta-lactamase enzymes and the results showed that a positive result of 28% as shown in Table 4. This indicates that bacterial resistance to antibiotics does not depend solely on its ability to produce beta-

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دراسة حساسية البكتيريا المعزولة ونتائجها لenantيمات بيتا لاكتاميز لبعض أنواع Staphylococcus spp. المولص

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الملخص

تتضمن الدراسة عزل بعض أنواع الجنس Staphylococcus الموجبة لصبغة كرام من عينات مختلفة تمثل (الادراز، مصحات الجروح، الحروق) من المرضى الراقدين في مستشفى الجمهوري التعليمي في مدينة الموصل لكل الجنسين ولفترة من تسعين الثاني سنة 2018 ومتتالية الاحصاء.

أظهرت النتائج عزل نوع واحد بكتيريا Staphylococcus aureus وكانت نسبة 13.2%، وأظهرت النتائج عزل نوع واحد بكتيريا Staphylococcus epidermidis و Staphylococcus capilis و Staphylococcus simulans و Staphylococcus chromogenesephiو Staphylococcus lentus.

أظهرت النتائج عزل نوع واحد المضاد Rifampin، Ciprofloxacin, Gentamycin, Pipracillin, Amikacin. في حين انتهكت نسبة المقاومة لجميع العزلات تناو مضادات المولص

عندنفوذ من خلال النتائج أن مضادات CIP كان الأشد تأثيراً على العزلات المولصة في الدراسة تناضت بالمضادات

وتمت تصنمل الدراسة التحري عن إنتاج أنتيمات بيتا لاكتاميز من قبل العزلات في الدراسة بالطرق البديلة (الأنبوب الأوزمي، البقعة الأبوبية، الأندوب الخلاصي) وبيبتيد النتائج عدم إنتاج الأنزيم باع طرقه من الطريقة البديلة، في 4 ملالات بكتيرية فيما ثبتت الأنواع في قابلية إنتاجها للأنزيم، وكانت نسبة الأنواع الأوزمي من أفضل الطرق للكشف عن إنتاج هذه الأنزيمات.

كما اختبرت نتائج الدراسة المولصة على نتائج أنتيمات البيتا لاكتاميز واسعة الطيف باستعمال طريقة National Committee Clinical Laboratory Standards الأولي (NCCL) وطريقة القرص المزدوج وقد أظهرت عزلة واحدة من كل من Staphylococcus capitis, Staphylococcus chromogenesephi و Staphylococcus lentus. فأنها تكون على إنتاج الأنزيم بطريقة القرص المزدوج أما بطريقة Ciprofloxacin, Gentamycin, Pipracillin, Amikacin. الردة على إنتاجها

National Committee Clinical Laboratory Standards