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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 sciuri* *Staphylococcus lentus* (*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 Ceftriaxone, 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 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 intersit 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 are 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 left to harden. Then incubated at 37 ° C for 24 hours to ensure there were no contamination and 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 Suceptibility 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 antibiotics 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 8.1×10^8 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) m 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, Pencillioic 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:

2.5² 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 cm³ of the red phenol solution and add 16.6 cm³ 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 μ L 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, ceftazidime, and cefepime. If the MIC values for these antibiotics

are higher than or equal to 2 µg/Cm³) 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 clavulin 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 epidermis*, *St. simulans* by 11.1% and 1 isolates of *Staphylococcus xylosus*, *Staphylococcus chromanogenes*, *Staphylococcus capilis* by 5.5%.

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

Bacteria	Number	Source of isolation	ratio
<i>Staphylococcus sciuri</i>	2	Urin	11.1
<i>Staphylococcus lentus</i>	2	urin wound	11.1
<i>Staphylococcus epidermis</i>	2	urin	11.1
<i>Staphylococcus simulans</i>	2	urin wound	11.1
<i>Staphylococcus xylosus</i>	1	Urin	5.5
<i>Staphylococcus chromanogenes</i>	1	Wound	5.5
<i>Staphylococcus capilis</i>	1	Wound	5.5
<i>Staphylococcus aureus</i>	7	Urin2,wound2,burn3	38.9

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].

Table (2) shows the percentages of bacterial isolates resistant to antibiotics

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

Ceftriaxone CRO, cloxacillin CX, ciprofloxacin CIP, Gentamycin CN, Piperacillin PRL, Amikacin AM, Rifampin RA, Erythromycin E, Nitrofurantoin F, Imipenem IMP, Ceftazidime CAZ, Cefixime CFM, Cefotaxime CTX, Oxacillin OX, Augmentin AMC

In table(2) the results shown that most isolates were resistant to various antibiotics. The results showed high resistance to cloxacillin, ceftriaxone, 100% resistance. The isolates showed high resistance to Augmentin, Cefixime and 95% and 89%, respectively. The results showed sensitivity to ciprofloxacin, Rifampin, Gentamycin, Amikacin, and Piperacillin. 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 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-lactase 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 - lactase 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 - lactase enzymes for three - way positive bacteria.

Bacteria	Acidimetric Methods	Iodometric Tube Method	Iodometric Spot Method
<i>Staphylococcus sciuri</i>	0	1	0
<i>Staphylococcus lentus</i>	0	1	1
<i>Staphylococcus epidermis</i>	0	1	2
<i>Staphylococcus simulans</i>	0	0	2
<i>Staphylococcus xylosus</i>	1	0	0
<i>Staphylococcus chromogenes</i>	0	1	0
<i>Staphylococcus capitis</i>	0	1	1
<i>Staphylococcus aureus</i>	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 - lactase enzymes

Bacteria	NCCLS Method	Double Disc Synergy Method
<i>Staphylococcus sciuri</i>	0	0
<i>Staphylococcus lentus</i>	0	1
<i>Staphylococcus epidermis</i>	0	0
<i>Staphylococcus simulans</i>	0	0
<i>Staphylococcus xylosus</i>	0	0
<i>Staphylococcus chromogenes</i>	0	1
<i>Staphylococcus capitis</i>	1	1
<i>Staphylococcus aureus</i>	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 المضادات الحيوية وانتاجها لانزيمات بيتا لاكتاميز لبعض انواع *Staphylococcus spp.* المعزولة من مصادر مختلفة في مستشفى الجمهوري التعليمي في مدينة الموصل

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الملخص

تضمنت الدراسة عزل بعض انواع الجنس *Staphylococcus* الموجبة لصبغة كرام من عينات مختلفة شملت (الادراج ، مسحات الجروح ، الحروق) من المرضى الراقدين في مستشفى الجمهوري التعليمي في مدينة الموصل لكلا الجنسين وللفترة من حزيران الى تشرين الثاني سنة 2018 وبمختلف الاعمار .

أظهرت النتائج عزل انواع بكتريا *Staphylococcus* بنسبة 13.2% وكانت بكتريا *Staphylococcus aureus* الأكثر شيوعاً من بين العزلات اذ تم الحصول على 7 عزلات بنسبة 38.9% تلتها بكتريا *Staphylococcus epidermidis* و *Staphylococcus sciuri* و *Staphylococcus simulans* ، *Staphylococcus lentus* ، *Staphylococcus chromogenes* ، *Staphylococcus xylosus* و *Staphylococcus scapilis* بواقع عزلة واحدة لكل منها بنسبة 5.5% .

تم التحري عن نسبة انتشار هذه البكتيريا حسب مصدر العزل وأظهرت النتائج ان اكثر العزلات تم تشخيصها من عينات الادراج اذ بلغ عدد العزلات 9 عزلات وبنسبة (50%) تلتها عينات الجروح وكان عدد العزلات 6 عزلات بنسبة 33% ثم عينات الحروق اذ بلغت عدد العزلات 3 عزلة بنسبة 17% وان النوع *Staphylococcus aureus* كان الأكثر سيادة في الادراج .

درست حساسية العزلات البكتيرية لـ 15 مضاداً حيويًا لمجاميع المضادات الحيوية المختلفة وبينت النتائج تفاوتاً في نسبة مقاومة العزلات لهذه المضادات إذ كانت مقاومة لمضادات Cloxacillin, Ceftriaxone وبنسبة 100% .

في حين انخفضت نسبة المقاومة لجميع العزلات تجاه مضادات Rifampin, Ciprofloxacin, Gentamycin, Pipracillin, Amikacin إذ لوحظ من خلال النتائج أن مضاد الـ CIP كان الأكثر تأثيراً على العزلات البكتيرية قيد الدراسة تلاه المضاد Rifampin .

وتضمنت الدراسة التحري عن إنتاج أنزيمات بيتا لاكتاميز من قبل العزلات قيد الدراسة بالطرائق الأيودية (الأنبوب الأيودية، البقعة الأيودية، الأنبوب الحامضية) وبينت النتائج عدم إنتاج الأنزيم بأي طريقة من الطرائق الأيودية من قبل 4 سلالات بكتيرية فيما تبينت بقية الأنواع في قابلية انتاجها للأنزيم وكانت طريقة الأنبوب الأيودية من أفضل الطرائق للكشف عن إنتاج هذه الأنزيمات .

كما اختبرت قابلية الأنواع البكتيرية على إنتاج أنزيمات البيت لاكتاميز واسعة الطيف باستعمال طريقة National Committee Clinical Laboratory Standards الاولى (NCCL) وطريقة القرص المزدوج وقد أظهرت عزلة واحدة من كل من *Staphylococcus lentus* و *Staphylococcus capilis* ، *Staphylococcus chromogenes* ، *Staphylococcus capilis* قابليتها على إنتاج الأنزيم بطريقة القرص المزدوج أما بطريقة NCCL الأولى فقد أظهرت عزلة واحدة لـ *Staphylococcus capilis* القدرة على إنتاجه .