مجلة الدراسات التربوية والعلمية - كلية التربية - الجامعة العراقية
العدد العشرون - المجلد الأول - علوم الحياة - تشرين أول 2022 م
doi.org/10.52866/esj.2022.01.20.20

Study the susceptibility of *Acinetobacter baumannii* production of Siderophore, isolated from different clinical specimens in Baquba

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Abstract:

In this study, (150) Specimen were collected from patients who were visiting or residing in Baquba General Hospital, Al-Batool Teaching Hospital and the Centers of Specialized Clinics for Respiratory and Pulmonary Diseases in Diyala province for the period from the 2nd January to the 2nd May 2021 after consulting the doctor.

These specimen collecting from site of infections as (60) of them were taken from the urinary tract, (30) from burns, (30) from wounds and (30) from respiratory tract infection.

The samples were sent to the laboratory and were subjected to microscopical, biochemical and cultural examinations and the final diagnosis was made using the API 20E system.

From different isolation sources, (9) isolates of *Acinetobacter baumanii* were obtained. Results of antibiotic sensitivity for bacteria to (9) antibiotics showed (100%) resistance to each of Amoxicillin, Cephotaxim, Trimethoprim and Aztreonam, and (80%) resistance to each of Gentamycin, Amikacin and Ciprofloxacin, while there was (40%) resistance to each of Imipenem and Augmentin, which is a mixture of the enzyme inhibitor (Amoxicillin and Clavulanic acid).

The minor inhibition concentration (MIC) was determined for the following (5) antibiotics: Gentamycin, Amikacin, Cefotaxime, Amoxicillin and Augmentin, which ranged between (<512-1024).

The ability of the isolates to produce Siderophores was also studied showing (100%) ability of the isolates to produce Siderophores.

دراسة مدى حساسية انتاج بكتيريا لحاملات الحديد المعزولة من عينات سريرية مختلفة *Acinetobacter baumannii*

الخلاصة:

جمعت 150 عينة من العينات السريرية من المصادر المختلفة من مستشفى بعقوبه العام ومستشفى البتول التعليمي ومراكز العيادات التخصصية للأمراض التنفسية والصدرية في محافظة ديالى للمدة من 2021/1/2 لغاية 2021/5/2 من المرضى المراجعين والراقدين في هذه المستشفيات والمراكز بعد استشارة الطبيب.

اخذت 60 عينه من التهاب المسالك البولية و 30 عينه من الحروق و 30 عينه من الجروح و30 عينه من التهاب المسالك التنفسية، تم ارسال هذه العينات الى المختبر وبعد اجراء الفحوصات المجهرية والكيموحيويه والزرعية والتشخيص النهائي بنظام ال API 20E .

تم الحصول على 9 عزلات تعود لبكتريا *Acinetobacter baumannii* من مصادر العزل المختلفة ، أظهرت نتائج اختبار الحساسية للبكتريا لـ 9 مضادات ووزعت ما بين Amoxicillin, Cephotaxim, Trimethoprim و Aztreonam بنسبة 100 ٪ و Gentamycin, Amikacin و Ciprofloxacin بنسبة 80 ٪ ، ومضاد Augmentin وهو خليط من مثبط الانزيم (Clavulanic acid وAmoxicillin) ومضاد Imipenem بنسبة مقاومة 40 ٪ .

وحُدد التركيز المثبط الادنى (MIC) لــ 5 من مضادات الحياة وهي Gentamycin وAmikacin و Cefotaxime وAmoxicillin و Augmentin وقد تراوحت هذه القيم للمضادات مابين (512 – 1024>)

درست قابلية العزلات على إنتاج حاملات الحديد (siderophore)إذ اظهرت العزلات قدرتها على انتاج حاملات الحديد (siderophore) بنسبة (100٪) .

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Introduction

Acinetobacter baumannii is one of the most pathogenic species. In recent years, it has been increasingly identified as an important human pathogen, causing acute nosocomial infections, as well as other infections, including urinary tract infections and pneumonia (1). These bacteria also cause respiratory tract infections, wound infections, septicemia, skin infections, soft tissue infections, endocarditis, meningitis (2).

Acinetobacter baumannii possesses many virulence factors that enable it to attack body tissues and cause infections. The first virulence factor produced by these bacteria is the siderophore, through which bacteria consume iron, because iron is an important element for bacteria and is not freely available in the host, but it is bound to heme, lactoferrin and transferrin molecules (3). Bacteria have the ability to live and proliferate under low iron conditions, whether in the environment or inside the host in the presence of these systems (4).

Several studies have reported the emergence of resistant bacterial strains to more than one antibiotic, which is considered a medical problem due to the difficulty of controlling diseases as a result of not choosing the appropriate treatment and the increasing and random use of antibiotics (5).

Materials and methods

Isolation of bacteria: In this study, (150) specimen was collected from different pathological conditions (wounds, burns, urinary tract infections and respiratory tract infections) from several hospitals in Diyala province including (Baquba General Hospital, Al-Batool Teaching Hospital and various public and private health centers).

Diagnosis of isolates: for diagnosis of isolates microscopic examination, culturing on Blood agar and MacConkey agar were used, as well as biochemical tests, such as Catalase, Oxidase and IMVIC test, while for the final diagnosis of the isolates, the API 20E system was used. The attached instructions for use by the manufacturer (Biomerieux) were adopted as stated by (6).

Antibiotic sensitivity test:

The antibiotic sensitivity test in the study was conducted according to the disc diffusion method or the (Kirby-Bauer method). Measurements of the inhibition zone were compared with the standard measurements of the Clinical and Laboratory Standards Institute CLSI 20 (7).

Determination of the minimum inhibition concentration (MIC):

The two-fold dilution method was used on the culture media agar to measure the minimum inhibitory concentration (MIC) of the antibiotics according to what was stated by (8).

Detection of Sidreophore :

The isolates to be diagnosed were grown on Iron-deficient minimal me-

dium and based on what was mentioned by (9).

Results and discussion

Sample collection

In the current study, (150) specimen was collected from patient in Baquba General Hospital, Al-Batool Teaching Hospital and the Centers of Specialized Clinics for Respiratory and Pulmonary Diseases in Diyala province (60 samples from urinary tract infections, 30 samples from burns, 30 samples from wounds and 30 samples from respiratory tract infections). After conducting microscopic, biochemical and culture examinations and carrying out the final diagnosis using the API 20E system, (9) isolates of *Acinetobacter baumanii* bacteria were obtained as shown in table (1) according to the isolate's sources.

Table (1): Distribution of *Acinetobacter baumanii* isolates according to isolation source

Isolation source	Number	Percentage
Urinary	2	22.22%
Respiratory	4	44.44%
Burn	2	22.22%
Wound	1	11.11%

Most of the infections caused by these bacteria occur in the respiratory and urinary systems, especially in the intensive care units (ICU) and surgery rooms, and this may be attributed to the ability of *Acinetobacter* bacteria to adhere to human epithelial cells in the presence of adhesion factors such as fimbriae and the capsules (10).

Also, the presence of mucus, which is a nutritional substance for bacteria, helps in their growth and proliferation, in addition to the lack of natural microorganisms that compete with pathogenic bacteria for food. The presence of large amounts of oxygen in the respiratory system, especially the lower part of it, encourages bacteria to settle and invade the area because they are obligate aerobic bacteria (11).

Our results were in agreement with the findings of (12) who reported that the highest percentage of *A. baumannii* isolates was from respiratory tract infections (sputum) 23(41.81%) isolates out of 55 isolates.

Our results disagreed with the study of (13) who showed the lowest percentage of bacteria was isolated from sputum samples, (12%) of a total of (66) isolates.

The result was also in disagreement with what was concluded by (14), who found that the highest percentage of isolates was from urine samples 5 (50%) isolates, and the lowest percentage of isolates was from burn and sputum samples (10% of the total 10 isolates) of *A. baumannii*.

The reason for the difference in the isolation percentage is due to the number of samples taken, the time of sample collection, the environment

from which the samples were isolated, the health conditions in which the patients live, the length of their stay in the hospital, the random use of antibiotics, the difference in the number of samples taken for the study, the variance in sample collection as well as the health conditions of the patients (15).

Diagnosis

The bacteria were initially diagnosed by microscopic examination then cultured on blood agar and Maconkey agar based on some differential characteristics of each type of bacteria. The colonies of *A. baumannii* bacteria that play an important role in causing urinary tract infections were circular, regular and pale because they are non-lactose fermenter, and some of their strains showed slightly pinkish color colonies because they slowly ferment lactose, but when they grow on blood agar, they appear convex, grayish-white and non-hemolytic (16), because they can't produce hemolysin (17). They are gram negative bacteria, immotile, Indole negative, methyl red negative and negative for Fox-Proskauer test, positive for citrate and negative for urease (18).

The sensitivity of the isolates to antibiotics

The sensitivity of the isolates from patients to (9) antibiotics was examined and showed in table (2). They were distributed among the beta-lactam group, which included Amoxicillin, Cephotaxim, Trimethoprim and Aztreonam, and the aminoglycoside group which included Gentamycin, Amikacin and Ciprofloxacin, Imipenem and Augmentin (a mixture of Amoxicillin and Clavulanic acid). It was based on measuring the diameter of the inhibition zone and comparing it with what was mentioned by (19).

Acinetobacter baumannii showed (100%) resistance to each of Amoxicillin, Cephotaxim, Trimethoprim and Aztreonam, and (80%) resistance to each of Gentamycin, Amikacin and Ciprofloxacin, while there was (40%) resistance to each of Imipenem and Augmentin, which is a mixture of the enzyme inhibitor (Amoxicillin and Clavulanic acid).

Table (2): susceptibility of isolates to (9) different antibiotics

Antibiotics	Resistance	
Amoxicillin, Cephotaxim, Trimethoprim and Aztreonam	No.	%
	9	100
Gentamycin, Amikacin and Ciprofloxacin	7	80
Imipenem and Augmentin	3	40

(20) stated that the increase in the spread of multi-drug resistant *Acinetobactor baumannii* is due to the excessive and random use of third-generation antibiotics, as the bacteria developed several resistance mechanisms, including the production of broad-spectrum beta-lactamase enzymes, modification in the target site of Penicillin-binding protein, and modification in purines as well as the presence of different types of beta-lactamase enzymes.

Studies have demonstrated that the cause of bacterial resistance to Cefotaxime and Ceftazidime antibiotics may be attributed to that these bacteria have genes that code for the TEM-2 beta-lactamase enzyme that destroys these antibiotics (21).

Our results were in agreement with the results of (22) in her study on *Acinetobactor baumannii* 40 local bacterial isolates, where there was (50%) resistance to Cefixime, Ceftazidime and Cefotaxime.

The results of our study showed that bacterial resistance to aminoglycoside antibiotics is because they have plasmids that code for the production of modified enzymes, which in turn prevents them from binding to ribosomes (22).

Our results also agreed with the study carried out by (23) who found that the bacteria showed (50%) resistance to Amikacin and (62.5%) resistance to Gentamicin.

The bacteria isolates under study showed a high resistance to the Quinolones group due to the presence of genes carried on conjugate plasmids or transposons that mediate resistance to anti-quinolones such as the aac(6)-Ib-cr gene carried on a plasmid that mediates resistance to anti-quinolones (24).

Studies carried out by (25) showed that there was (100%) resistance to Ciprofloxacin.

Bacterial isolates showed high resistance to anti-Trimethoprim, and this may be due to their possession of efflux pumps and a mechanism to change the permeability of the plasma membrane (26), and the study agrees with (27) who showed that there was a (81%) resistance to the antibiotic.

Our findings agreed with the study carried out by (28) who showed that the resistance rate to Imipenem was 50%.

Determination of minimum inhibitory concentrations (MICs) to antibiotics

The minimum inhibitory concentrations of the most antibiotic-resistant bacteria that resisted to 9 antibiotics were studied for several causes, including its high accuracy and the importance of this test in medicine and treatment of bacterial infections. Most of the isolates showed high resistance to the antibiotics used at concentrations that were multiples of the break points. The sign (>) was used with the recorded minimum inhibitory concentration values to indicate continued growth despite the

large concentrations used. The isolates were determined as sensitive or resistant to the antibiotic depending on the break point, which was established by (18) as a basis for calculating the response and represents the optimum concentration that the antibiotic can reach in the serum so as to provide the maximum concentration of treatment. The isolate is said to be sensitive when the MICs are less than the calculated break point in µg/ml. The MIC values of the local isolates were determined by the Serial dilution concentrations on Muller-Hinton agar medium, as there are several factors can affect the MICs values, including the components of the medium used, which clearly affect the calculated MICs. Therefore, it is preferred to use Muller-Hinton agar because it contains a little amount of sodium chloride and a small concentration of calcium and magnesium ions (8). The MIC values are also affected by the size of the bacterial inoculum, as these values increase with the increase in the size of the bacterial inoculum.

In this study, (5) types of antibiotics were used: Gentamycin, Amikacin, Cefotaxime, Amoxicillin and Augmentin.

The results indicate that the minimum inhibitory concentrations (MIC) of the Amoxicillin ranged between (512-1024) to *A. baumannii* bacteria. The high resistance to Penicillins, which include (Amoxicillin and Paracillin), is due to several mechanisms, such as the destruction of the antibiotic by beta-lactamase enzymes or the failure of the antibiotic to penetrate and reach the target site (penicillin binding protein PBPs), or reduce the affinity of the antibiotic to bind with the PBPs (29).

Regarding Augmentin, the MIC value was (1024) for the isolates as well. This resistance is attributed to the high production of ESLs by the bacteria, due to genetic mutations in the gene encoding the production of this type of enzyme that leads to the replacement of amino acids by others, resulting in bacterial resistance to antibiotics (30).

The minimum inhibitory concentration (MIC) of Gentamycin was (1024) for *Acinetobacter baumanni*, while the MIC value of Amikacin was (512).

The high resistance may be attributed to the production of aminoglycoside-modifying enzymes which modify the common amine or hydroxyl group and thus produce antibiotics with weak binding to ribosomes, or it may be attributed to changing the binding site of the ribosome to the antibiotic (31).

Detection of siderophore production

The results showed that there was (100%) production of siderophores by the bacteria, and this result coincided with the results of (32), who found that the production of bacteria to siderophores was (99%). Our results also agreed with (33) who found (100%)

production of siderophores, and (34) who indicated the ability of all isolates to produce siderophores, which confirms their role in the pathogenicity of these bacteria.

It should be mentioned here that all isolates capable of producing the sidrophore system are unable to produce hemolysin on blood agar medium. It is clear that there is a relationship between hemolysin and siderophore production, which confirms that the non-hemolysin producing intestinal bacteria that grow on blood agar can produce siderophores in order to be able to chelate iron from its compounds for their necessary need for it.

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