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Identification of *Acinetobacter baumannii* Isolated from Different Infections and Study the Prevalence of Antibiotic Resistance in Patients of Baquba City

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Abstract

Acinetobacter baumannii one of the important multidrug-resistant (MDR) opportunistic nosocomial pathogens, in part due to its high capacity of acquiring resistance to diverse antibiotic groups. Twenty five Clinical sample samples were gathered from various sources from Baquba Teaching Hospital, from the period june2022 to October 2022 after consulting with the expert doctor, from both in- and out-patients, and the samples were sent to the lab. Each sample's data was entered onto a unique form, and they included: 10 (sputum samples from respiratory tract infections; 5 burn samples; 4 wound samples; 6 samples for urinary tract infections. After being cultured on MacConkey agar and Blood agar, the isolates were identified. Oxidase and Catalase biochemical tests were performed on the samples, and Vitik2 was employed to make the final diagnosis. Twelve isolates of Acinetobacter baumannii were found after culture and identification; of these, five were found in sputum, two in burns, two in urinary tract infections, and three in wounds. Results of 10 different antibiotics' sensitivity tests on A. baumannii isolates from various clinical sources at the Baquba Teaching Hospital showed that the bacterium showed varying degrees of resistance to the medications.. 100% of the samples displayed resistance to Cefoxitin, Oxacillin, Ceftazidim, Cefepime, and Tetracyclin, while 75% displayed excellent susceptibility to Imipenem, The resistance patterns of isolates

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were also determined isolates showed to quinolone antibiotic such as Levofloxacin 6/12(50%), Norfloxacin 7/12 (58.33%) , Ofloxacin 6/12(50%) ,Ciprofloxacin 3 /12(25%).

Keywords: *Acinetobacter baumannii*, Vitik2, Antibiotics ,quinolone, Imipenem,Oxidase

تشخيص بكتريا الراكدة البومانية المعزولة من اصابات مختلفة ودراسة انتشار مقاومتها للمضادات الحيوية في مرضى مدينة بعقوبة

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الخلاصة

تم جمع 25 عينة سريرية من مصادر مختلفة من مستشفى بعقوبة التعليمي ، من الفترة من حزيران2022 إلى تشرين الاول 2022من مصادر سريرية مختلفة وباشرافالطبيب الاختصاصي من المرضى الداخليين والخارجيين للعيادات الخارجية وعيادات الاطباء ، وتم إرسال العينات إلى المختبر. تم إدخال بيانات كل عينة في نماذج لاستمارات موحدة ، وتضمنت العينات : 10 (عينات من القشع من التهابات الجهاز التنفسي ؛ 5 عينات حروق ؛ 4 عينات جرح ؛ 6 عينات لالتهابات المسالك البولية). تم التعرف على العزلات بعد زراعتها على وسط الماكونكي اجار وأجار الدم. وكذلك تم إجراء اختبارات كيميائية حيوية على العينات مثل اختبار الاوكسديز واختبار الكاتاليز ، وتم استخدام 2Vitik لإجراء التشخيص النهائي. تم الحصول على اثني عشر عزلات من Acinetobacter baumannii. من بين العينات ، حيث تم الحصول على خمسة عزلات في القشع ، واثنان في الحروق ، واثنان في التهابات المسالك البولية ، وثلاثة في الجروح.و أظهرت نتائج اختبارات الحساسية للمضادات الحيوية حيت تم اختيار عشرة مضادات حيوية مختلفة على عزلات A. baumannii ، أن درجات المقاومة المختلفة للمضادات الحيوية العشرة متفاوتة.و أظهرت النتائج ان 100٪ من العينات مقاومة لمضادات Cefoxitin و Oxacillin و Ceftazidim و Cefepime و Tetracyclin ، بينما أظهر 75٪ حساسية ممتازة تجاه عقار Imipenem ، كما تم تحديد أنماط مقاومة العزلات للمضادات الحيوية من نوع كوينولون وكانت النسب Levofloxacin 12/6 (50٪)) ، نورفلوكساسين 12/7 (58.33٪) ، أوفلوكساسين 12/6 (50٪) ، سيبروفلوكساسين 12/3 (25٪).

الكلمات المفتاحية : الراكدة البومانية , فايتك 2، مضادات حيوية ،، اوكسيديز ، الكوينولون . الامبيبنيم .

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Introduction

The gram-negative coccobacillus *Acinetobacter baumannii*, which was once thought to be an opportunistic pathogen, is now recognized as a significant contributor to healthcare-associated illnesses. Most recently, A.baumannii has become resistant to most effective antimicrobial agents and causing a high incidence rate of morbidity and mortality especially in the intensive care unit in many countries [1]. *Acinetobacter baumannii* strains have the ability to colonize several ecological niches including soil, water, and animals, including humans. *Acinetobacter baumannii* one of the important multidrug-resistant (MDR) opportunistic nosocomial pathogens, in part due to its high capacity of acquiring resistance to diverse antibiotics groups [2]. Different mechanisms play a role in the acquisition of multidrug resistance (MDR) phenotype among *Acinetobacter baumannii* strains. This is because they possess a wide range of genes that they are encoded to antibiotic resistance, both intrinsic and acquired [3]. The most common feature is the capacity to manufacture carbapenemase enzymes such oxacillinases (Ambler class D OXA-type) and metallo—lactamases (Ambler class B). They thrive on inaccessible and durable carbon molecules.which helps them live in adverse environmental conditions [4]. The causative agent for bloodstream infections, urinary infections, wound infections, and ventilator-associated pneumonia is commonly identified as *Acinetobacter baumannii* [5].There are three types of antibiotic resistance mechanisms of *A. baumannii* are as follows: First, by decreasing membrane permeability or boosting antibiotic efflux, resistance can be created, blocking access to the target. Additionally, bacteria may use genetic change or post-translational alteration to defend the drug target, and ultimately, antibiotics can be inactivated directly through hydrolysis or modification process [6]. This pathogen developed multidrug resistance (MDR) in recent years, primarily as a result of widespread antibiotic overuse and poor antibiotic management. Long hospital stays, catheter use, and mechanical ventilation are related with MDR isolates, and invasive infections are more likely to occur in immunocompromised and critically unwell hosts [7].

Aim of Study: Isolation and identification of *Acinetobacter baumannii* from different clinical infections. Investigations of the occurrence of multi-drug resistant and antibiotic susceptibility profile in *Acinetobacter baumannii* isolates.

Material and Methods

1. Sample collection

Twenty five clinical samples were collected from different sources from Baquba teaching hospital and Specialized Clinics for Respiratory and Chest Diseases in the city of Baquba for a period of june2022 Until October 2022, the cases of the patients who returned or were hospitalized in these hospital after consulting the specialist doctors. Data from each sample were kept on unique forms and they comprised 10 respiratory tract infection samples 5 samples of burns, 4 samples of wounds, and 6 samples of UTI. To choose the necessary microorganisms, it was cultivated on the culture media.

2. Isolation and Identification of *A. baumannii*

The collected specimens were inoculated on the Maconkey and blood agar, incubated at 37ºC for 24 hours. The isolates were examined for their shape, size, color, pigments, and hemolytic activity. Then transferred and streaked on Pseudomonas base agar. All plates were incubated at 37ºC for 24 hours then a single pure isolated colony was transferred to Nutrient agar medium for the preservation and to carry out other biochemical tests that confirmed the identification of isolates. The isolates were identified according to the Bergey's Manual [9]. as the following: gram stain and biochemical tests which included (catalase test, oxidase test, IMVC and TSI test).The device uses the VITEK 2 to conduct biochemical tests for bacterial isolates. This device includes 48 tests of biochemical tests that are used in the diagnosis of germs, so that the accuracy of diagnosis in this device is 99%.

3. Antibiotic sensitivity test

The isolates were tested for the sensitivity of the antibiotics used in the study, according to the method of diffusion by plate or what it is also called the Bauer-Kirby method. Measurements

of the size of the inhibition band were compared with the measurements documented by re-standards.Clinical and Laboratory International 2017 CLS (9). As shown in Table (1)

Table 1: Antibiotics used in the study with their concentrations

Antibiotic class	Antibiotic name	Symbol	Concentration µg/disk	Zone diameter (mm)(CLSI,2017)		
				S	I	R
β-lactams / Penicillins						
	Oxacillin	OX	1	24	19-23	18
β-lactams / Cephems						
	Cefoxitin	FOX	30	22	-	21
β-lactams /Cephalosporins						
	Ceftazidime	CAZ	30	20	17-19	16
	Cefepime	FEP	30	29	24-28	23
β-lactams / Carbapenems						
	Imipenem	IMP	10	29	24-28	23
Quinolones / Fluoroquinolones						
	Ciprofloxacin	CIP	5	21	20-16	15
	Norfloxacin	NOR	10	17	16-13	12
	Levofloxacin	LEV	5	19	16-20	15
	Ofloxacin	OFX	5	18	15-17	14
Tetracyclines						
	Tetracycline	TE	30	19	15-18	14

4. Statistical analysis

Analysis of data was carried out using the available statistical package of SPSS- (Statistical packages for Scial Sciences –version).

Results and Discussion

1. Sample collection and Distribution

All the isolates were collected from different clinical sources grown on primary isolation and selective media and subjected to Gram's staining, catalase and oxidase tests in addition to other biochemical tests. After being cultured on MacConkey agar and Blood agar, the isolates were

identified. Growth Due to the non-lactose fermenting colonies' lack of pigmentation, tiny size, and regular edges, MacConkey agar displayed a light pinkish hue.The colonies of *A.baumannii* isolates on blood agar appeared white to cream-colored, smooth and circular with entire edges. Most species were non-hemolysis. Colonies became more mucoidal upon further incubations [10]. Found, and Vitik2 was utilized for the final diagnosis. Twelve isolates of *Acinetobacter baumannii* were found after culture and diagnosis as shown in table (2)

Table 2: Identification of *Acinetobacter baumannii* by biochemical test

NO	Biocheical test	Result
1	Gram stain	Negativ
2	Microscopic shape	Coccobacilli
3	Growth at 44°C	Positive
4	Lactose fermentation	Negative
5	Hemolysin production	- γ hemolysis
6	Oxidase test	Negative
7	Catalase production test	Positive
8	Methyl red	Positive

These tests allowed for a quick identification of the unidentified isolate based on the color variations that occur in the various assays. After 24 hours at 37°C. *A. baumannii* isolates passed In the case of the catalase test after adding a hydrogen peroxide reagent to colonies, all of the suspected isolates produced gas bubbles, indicating a good result for this test. However. The oxidase test returned negative results. Because they were exclusively aerobic, they provided an alkaline/acid kind of growth slant in the Kliglar iron agar test, did not modify the bottom, and produced no H2S without producing any gas. None of the isolates produced indole, according to biochemical testing. The red methyl test and vogus proskauer test also returned negative findings. The positive results for the test appeared in Simmons citrate, while the urease test gave variable results. These common biochemical tests were conducted in the current investigation, and the results were compared with the standard result documented by [10]. Acinetobacter is easily isolated in typical cultures but is relatively nonreactive in many biochemical tests that are usually used to distinguish among gram-negative bacilli [11]. All isolates of *A.baumannii* had the ability to grow at 44°C and were positive on nutrient agar media. The isolates can produce heavy growth after 24 hrs of incubation. This test was used to

differentiate *A.baumannii* from other Acinetobacter species reading the ability to grow at this temperature degree. This is considered the best method to identify *A. baumannii* from other bacteria [12]. Distribution of *A.baumannii* among clinical samples was different according to the source and percentage of isolation, it could be said the percentage of *A. baumannii* among the clinical samples were varied According to the source of the samples. The results shown in table (3) appeared that from the total 12 isolates 3(75%) and 5(50%) isolated from wound infection and sputum respectively. The lowest number and percentage of *A. baumannii* was UTI 2(33.3%) and burn infection 2(40%).

Table 3: Distribution of Acinetobacter *baumannii* isolates according to isolate sources, Gender

	UTI			Sputum			Wounds			Burns		
	Total	A. baumannii	% positive	Total	A. baumannii	% positive	Total	A. baumannii	% positive	Total	A. baumannii	% positive
Male	4	1	25%	6	4	66.7%	3	3	100%	2	1	50%
Female	2	1	50%	4	1	25%	1	0	0%	3	1	33.3 %
Total	6	2	33.3 %	10	5	50%	4	3	75%	5	2	40%

The increase of *A. baumannii* in wounds due to its ability to biofilm formation in the skin and soft tissue infections, as well as in occlusive dressings [7]. A bacteriological study stated that more than 60% of hospital-acquired pneumonia infections are caused by Negative bacteria including *Acinetobacter baumannii* the reason for this may be attributed to their ability to blind adhesion to epithelial cells in the respiratory tract as a result of inhibitory substances such as bacteriocins, wa as the presence of mucous matter [10]. The presence of an ample amount of oxygen in the respiratory tract, especially the lower part of it, encourages bacteria to settle and

invade the region because it is a forced wind [13]. The ability of the organism to survive in a hospital environment for an extended period of time, develop antibiotic resistance quickly, and propagate clonally all contribute to the species' persistence and transmission in healthcare settings. As a result, *A. baumannii* has the potential to cause a wide range of serious nosocomial infections, including ventilator-associated pneumonia, wound infections, bloodstream infections, meningitis, and urinary tract infections. Debilitating patients, those admitted to intensive care units (ICUs), those with indwelling foreign devices, and those who are mechanically ventilated are among the high-risk categories [14][15].The one of the causes urinary tract infections (UTIs), especially with indwelling urinary catheters. Moreover, it is unusual for this organism to cause uncomplicated UTI in healthy outpatients [16]. As for the local studies, the results partially agreed with what was reached by the researcher [17] who found that the highest isolation rate was obtained from sputum samples 41.66 of the total 12 isolates, and the result differed with what the researcher reached [18] Which found that the highest percentage of isolation was from urine samples 5 isolated 50% and the lowest percentage in burn and sputum samples 10%from a total of 10 isolates of *A. Baumannii*. Burns remain a major global public health problem in terms of long-term disability, morbidity and mortality, particularly in the developing countries [19]. Many bacterial species harbor specific receptors for such molecules and, hence, burn wound surfaces are easily colonized by bacteria The reason for the difference in the isolation ratio is due to the number of samples taken the time of collection of the samples, the environment from which the samples were isolated, the health conditions in which the patients lived, and the length of time they stayed in Hospitalization, indiscriminate use of antibiotics excessively and the difference in the number of samples taken for a study and variance.

2. Resistance of bacterial isolates to antibiotics

The results of the *A. baumannii* sensitivity tests for isolates from diverse clinical sources from the Baquba Teaching Hospital showed that the bacterial isolates were resistant to various antibiotics at varying rates. All samples (100%) showed resistance to Cefoxitin, Oxacillin, Ceftazidim, Cefepime, and Tetracyclin, while 75% displayed excellent susceptibility to

Imipenem, The resistance patterns of isolates were also determined isolates showed to quinolone antibiotic such as Levofloxacin 6/12(50%), Norfloxacin 7/12 (58.33%) , Ofloxacin 6/12(50%) ,Ciprofloxacin 3 /12(25%).in fig (2).

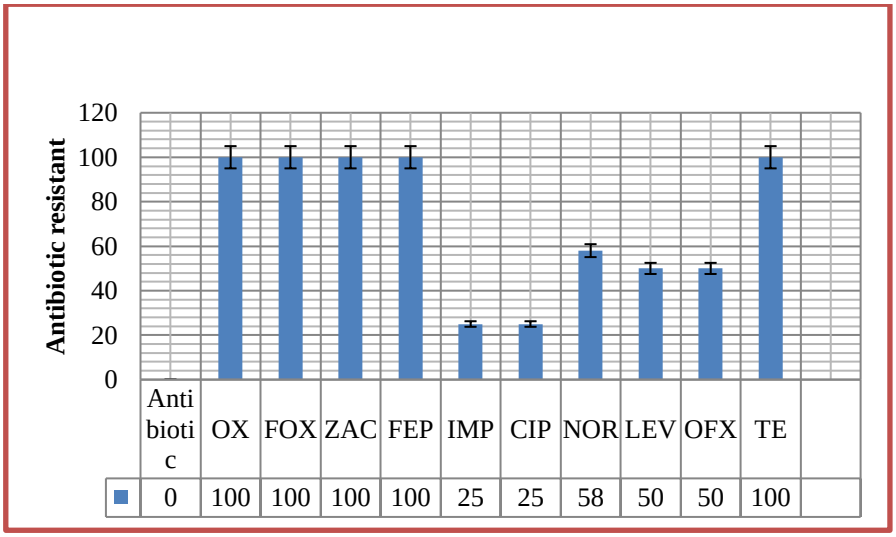

Figure 2: Resistance rates of *A. baumannii* isolates to 10 antibiotic in the study

The results of (Imipenem) which considered as drug choice of treatment with (75% percentage sensetive). The resulting disagreement to study of the neighboring countries, such as Turkey (98%) and Pakistan (100%), and Iran(90%)and Saudi Arabia 90% [20][21][22][23].

The results of the current study demonstrated that the highest resistance to almost all β-lactam antibiotic classes understudy was as follows cephalosporins (Cefoxitin , Cefoxitin, Oxacillin, Ceftazidime, Cefepime) showed complete resistance with a percentage rate of 10(100%), These findings were close to local studies related to *A. baumannii* isolates by [24], who found that (100%) of *A. baumannii* isolates in Iraqi hospital environment resisted Oxacillin, Ceftazidime and Cefepime. Another study in Iran hospital by [25] found (100% resistance rate to (Cefoxitin , Cefoxitin, Oxacillin, Ceftazidime, Cefepime) .

The highest antibiotic resistance rate that *A. baumannii* isolates exhibited was to tetracycline 12(100%). The result of the present study disagreed with study done by [26] who showed that resistance rate of these isolates to tetracycline was (62.8%). The results of this study agreed

with India's study [27] who mentioned that the complete resistance to Tetracycline (100%) . The resistance mechanisms are by efflux and ribosomal protection that cause resistance in older-generation Tetracycline [26] .While the mod of action of the Tetracycline group inhibits bacterial protein synthesis by binding to the 30S ribosomal subunit [27].

The resistance patterns of isolates were also determined as the *A. baumannii* isolates showed same levels of resistance to quinolone antibiotic such as Levofloxacin 6/12(50%), Norfloxacin 7/12 (58.33%) , Ofloxacin 6/12(50%) ,Ciprofloxacin 3 /12(25%). The finding that almost supports a previous local study by [18] who demonstrated the resistance of *A. baumannii* to Quinolone was 30% for Ciprofloxacin and far away from resistant to Norfloxacin (20%) . Some evidence hypotheses that efflux pumps can be used by the cell as a first line defense mechanism that prevents drugs from reaching the cellular lethal concentrations[28].The diversity of fluoroquinolone antibiotics, mainly Ciprofloxacin, made the effective treatment of infections caused by *A. baumannii* strains feasible, but these strains rapidly become resistant to these antimicrobial agents[29] Fluoroquinolone resistance is frequently brought on by changes to DNA gyrase or topoisomerase IV's structural components as a result of mutations in the quinolone resistance-determining areas[11]. These antibiotics are widely used in the treatment of *A. baumannii* infections in Baquba hospitals. As a result of this wide spread use, the level of resistance of *A. baumannii* was high in this study.

Infections caused by multidrug-resistant (MDR) microorganisms are correlated with high mortality compared to those caused by susceptible bacteria and carry important economic burdens [30].Other reasons could be due to the increase in irrational consumption rate of antibiotics self-medication, non-compliance with medication, the spread of resistance isolates among individuals, and the sale of subpar medicines. It is also obvious that regional customs have an equal impact on the exponential expansion of resistance, as the majority of individuals turn to purchasing prescription medications from pharmacies without the proper prescription, shelf life [31].

Conclusion

1. Acinetobacter baumanii had demonstrated widespread resistance to many modern antibiotic
2. Imepenem and Ciprofloxacin are the best drug of choice of Acinetobacter baumanii isolated according to the susceptibility test
3. All isolates of *baumannii* bacteria were shown. 100% were resistant to Cefoxitin, Oxacillin, Ceftazidim, Cefepime, and Tetracyclin.

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