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دراسة جزيئية لبكتريا *Acinetobacter baumannii*- المعزولة من عينات سريرية مختلفة في مدينة بعقوبة

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وهي جزء من متطلبات نيل درجة الماجستير في علوم الحياة
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Introduction :

   Acinetobacter species consist of gram-negative coccobacilli bacteria that are widely occurring in nature with studies showing that the genus can be isolated from soil and surface water samples (Baumannm *et al* .,1968; Peleg *et al*.,2008) the most medically important species, *Acinetobacter baumannii* was found to rarely occur on human skin under normal statuses (Dijkshoorn *etal*.,2005) Despite that it was described increasingly to cause nosocomial skin and soft tissue infections which can be fatal (Barbier *et al*.,2020; Motbainor *et al*.,2020)   It is opportunistic pathogen, has a high occurrence among immunocompromised individuals, particularly those who have experienced a prolonged hospital stay (Montefour *et al*., 2008).. The World Health Organization (WHO) had acknowledged that the resistance of *Acinetobacter baumannii* as one of three most important problems facing human health (Bassetti, 2011). The isolates of *A. baumannii* that isolated   from clinical samples were commonly resistant to multiple antimicrobial drug classes and have the ability to survive in the environment for along time. (Mohd *et al*., 2018).

   Acinetobacter baumannii has become a major cause of infection in the conflict regions, and has gained particular notoriety in the recent desert conflicts in Iraq, earning it nickname "Iraqibacter" Especially, high incidences of Multi Drug Resistant (MDR) bacteremia which was bloodstream infections have been determined among US Army members (Howard *et al*.,2012).

   In the past years *A. baumannii* strains have been increased significantly ,these isolates are often multidrug-resistant (MDR) (Perez *et al*., 2007). *A. baumannii* MDR isolates have a devastating ability to persist and circulate in the hospital environment, and their ability to induce biofilm formation on both

abiotic and biotic surfaces is beneficial for this, due to the surface colonization of hospital equipment and medical devices, such as urinary catheters, central venous catheters, endotracheal tubes, etc. (Dijkshoorn *et al*., 2007).

The factor involved in bacterial resistance to diverse antibiotics, survival in hospital environments, and chronic infections is the biofilm formed ability by *A .baumannii*. The Previous studies have found a positive relationship between the antibiotic resistance and biofilm formation in *A. baumannii* strains. There are also different of virulence factors, including the biofilm-associated protein (BAP), Chaperon-Usher pilus (Csu), outer membrane protein A (OmpA), and quorum-sensing system, that are involved in the biofilm formation of *A. baumannii*. The BAP encoded by the *bap* gene has a critical function in the biofilm realization and intercellular coalescence (Th

ummeepak *et al*., 2016; Fattahian *et al*., 2011). The *csuE* gene plays a major role in *A. baumannii* biofilm formation because the ability of *A. baumannii* to form biofilm is largely dependent on pili (Tomaras *et al*., 2008). In the worldwide spreading of *A. baumannii* in the hospitals has increased vividly. Therefore, using molecular typing methods is essential for epidemiological studies and infection control based on the epidemiological studies.(Diancourt *et al* .,2010; Pourcel *et al*.,2011) However ,there are other mechanisms made *A,baumannii* MDR isolates such as carbapenem resistance which was gradually became a great source of worry essentially among the nosocomial strains that belong to (ESKAPE) group of the pathogenic *species* (Bush *et al* .,2010), and CRAB produce of carbapenemases as a result of the carbapenem hydrolyzing class D β-lactamases. The OXA type carbapenemases of class D β-lactamases are divided in to five subgroups, four of them are acquired carbapenemases, which include: (*oxa- 23, oxa-24,*

oxa-58 , oxa-143),and the fifth sub groub is (*oxa*-51) which only one fundamental to *A. baumannii* (Opazo *et al* .,2012). The (*bla*oxa-23 gene) is one of the most predominant β-lactamase genes on the genome (which was mostly on the plasmids) of carbapenem-resistant *A. baumannii*. Rapid and specific of identification of *A. baumannii* and the strains harboring(*bla*oxa-23 gene), will proposition referential information on the control defenses and therapeutic for the nosocomial infections owing to the (CRAB) (Peleg *et al*.,2008; Yamamoto *et al*.,2015)

The Aims of this study :

1. Isolate of *Acinetobacter baumannii* from different clinical sources, and identified the isolates by phenotypic and genotypic methods.

2. Study the antibiotic susceptibility profile in *Acinetobacter baumannii* isolates, and investigate the occurrence of multidrug resistant isolates ,In addition to the minimum inhibitory concentration (MIC) for certain antibiotics.

3. Phenotypic detection of the Extended –Spectrum B- lactamase (ESBLs) and Metallo beta-Lactamase (MBLs) enzyme.

4. Molecular detection of some B-lactamase resistance genes (*bla*oxa23, *bla*oxa51*, bla*IMPand *bla*NDM-1) and molecular detection of some biofilm formation genes (*bap* and *csuE* genes).

5. Study the gene expression of *bap* and *csuE* by   *bla*oxa 51 as a house keeping gene using quantitative RT-PCR technique.

6. Studies the effect of low and high concentration of iron on biofilm formation to isolates which have the highest titer of biofilm formation using gene expression .

الخلاصة

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الراكدة البومانية *Acinetobacter baumannii* هي بكتريا سالبة لصبغة كرام انتهازية مرتبطة بالعدوى المكتسبة من المستشفيات، من غير شك انها مقاومة للمضادات المايكروبية و القدرة على تكوين الغشاء الحيوي من قبل العزلات السريرية التي تمتلك النظم التي تمكنها من البقاء في بيئات المستشفى واستعمار الادوات الطبية . خلال الفترة الزمنية من شهر أيلول (2020) ولغاية شهر اذار (2021) ، تم جمع 220 عينة من مصادر سريرية مختلفة (الدم، ،الحروق ،الجروح ،الادرار ،القشع، الصديد، والسوائل) . أظهرت النتائج ظهور نمو بكتيري في 138(62.7 %) من هذه العينات, و 82 (37.3 %) عينة كانت سالبة النمو. ومن بين موجبة النمو كانت 22 عزلة (10 %) عائدة للراكدة البومانية .

التشخيص الاولي للراكدة البومانية *A.baumannii* تم من خلال الماكونكي اكار بالاعتماد على خصائص الزراعة و الفحوصات البايوكيميائية كذلك من خلال نظام VITEK 2 Compact system GN وتم اجراء التشخيص الجزيئي جينات *bla*oxa51 و 23 *bla*oxa وفقا لفحص تفاعل البوليميراز المتسلسل PCR كانت نسب النتائج الموجبة على التوالي (100، 81 %) من المجموع الكلي لعزلات الراكدة البومانية *A.baumannii*. كما ان اظهرت النتائج 22 عزلة للراكدة البومانية *A.baumannii* كانت معزولة من: الحروق11 (50%)،الدم 5 (22.7%),الجروح 3 (13.6%) الادرار2 (9.1%)السائل الدماغي الشوكي 1 (4.6%).

تم أجراء فحص الحساسية بطريقة انتشار القرص (Kerby Bauer method)ضد12مضاد حيوي حيث ابدت العزلات مقاومة عالية (100%) ضد المضادات الحيوية (Amikacin, Ciproflaxacine Levoflaxacin and) بينما المقاومة ضد (Ampicillin sulbactam, Ceftraxon, Doxycylin,Impenem, Meropenem,Trimetheprim Sulfomethazol and Ceftazidme)كان على التوالي (81.8% and 77% , 90.9,%, 86%, 40.9%, 90.9%, 95%)كما اظهرت جميع العزلات مقاومة مقدارها صفر (0%) لكل من colistin وpoly myxin B. بحسب نتائج فحص الحساسية صنفت العزلات الى فئتين (27.3%) MDR ،(72.3%)XDR .

تم اختبار التركيز المثبط الادنى MIC لجميع العزلات بأستخدام ثلاث مضادات هي(Ceftazidum , Colistinو levofloxacine) بأستخدام الطريقة المعيارية بواسطة لوحة العيار الدقيقة وكانت قيمة