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Molecular Detection and Gene Expression for *hcp* and *bla*$_{OXA-51}$ Genes in *Acinetobacter baumannii* Isolated from Different Clinical Sources

A thesis

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By

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2. Literatures Review

2.1.History of *Acinetobacter baumanii*

The history of the genus Acinetobacter dates back to the early 20th century, in 1911, when Beijerinck, a Dutch microbiologist, isolated a microorganism from the soil by enrichment in minimal medium contains calcium acetate (Dijkshoorn, 2008). A major breakthrough in the long and complicated history of the genus was achieved in 1986 by Bouvet and Grimont, who—based on DNA-DNA hybridization studies—distinguished 12 DNA (hybridization) groups or genospecies, some of which were given formal species names, including *A. baumannii*, *A. calcoaceticus*, *A. haemolyticus*, *A. johnsonii*, *A. junii*, and *A. lwoffii*. In 1971, the sub-committee on the Taxonomy of Moraxella and Allied Bacteria officially acknowledged the genus *Acinetobacter* based on the results of Baumann's 1968 publication (Howard *et.al*,2012).

Acinetobacter baumannii is one of the most strains that during the past three decades has emerged from an organism of questionable pathogenicity to an infectious agent of importance to hospitals worldwide. Clinically the most important of 25 *Acinetobacter* genospecies, increased from 9% in 1995 to 40% in 2004 (Munoz-Price *et al*.,2008). Bacteriological studies on the antibiotic resistance in *A. baumannii* in Iraq are relatively new; the first published paper appeared in 2001. Since then, more data have been made available and the real situation of antibiotic resistance in hospitals is alarming (AL-Marjani *et al*,2016).

2.2. Taxonomy

This Bacteria was classified as members of the genus *Acinetobacter* have a long history of taxonomic changes, moving from the family

Neisseriaceae to the family Moraxellaceae. Within the genus Acinetobacter, studies based on DNA/DNA hybridization have resulted in the description of 25 “genomic species” that fulfilled the criteria to be considered distinct species(Fournier(a) *et al*.,2006).

The *Acinetobacter* genus currently comprises 34 species, with the *Acinetobacter* calcoaceticus-Acinetobacter baumannii (ACB) complex including four phenotypically related species: *A. baumannii*, *A. calcoaceticus*, *A. pittii*. Advances in molecular genotyping and whole genome sequencing have made possible delineation of individual Acinetobacter species within clinical isolates of the ACB complex (Fitzpatrick *et al*.,2015).

The species’ names have endured substantial taxonomic changes over the years due to the advanced understanding of molecular methods of the genetic make-up of this group of microorganisms. Recent classifications that seem to have gained wide acceptance among bacterial taxonomists have accepted this group of bacteria as gamma Proteobacteria categorized in the order Pseudomonadales and the family Moraxellaceae. Thus the taxonomical classification is given as; Domain: Bacteria, Phylum: Proteobacteria, Class: Gamma Proteobacteria, Order: Pseudomonadales, Family: Moraxellaceae, Genus: *Acinetobacter*. The species *A. baumannii*, *Acinetobacter haemolyticus* and *A. calcoaceticus* are of clinical significance (Almasaudie,2018).

2.3. General characteristics:

The Maine characters on the most study of *A. baumannii* is a Gram-negative bacillus that is aerobic, pleomorphic, non-fermenting, non-fastidious,non-motile, catalase-positive, oxidase-negative, and the bacteria with a DNA G + C content of 39% to 47% (Howard, *et.al*,2012). Even though they are non-motile, some "twitching" or "gliding" on semisolid media has occasionally been reported; This may be due to the activity of type IV glucose-6-phosphate isomerase (*gpi*) and RNA polymerase 70 factors (*rpoD*) (Bartual *et al*.,2005).

The bacteria carries ISAba 125 gene and ISAba1is one of important insertion element present upstream gene, and that is significant requires to act as a strong transcriptional promoter for *bla*$_{\text{OXA-51}}$ like gene, the last responsible for hydrolyzing penicillins (benzylpenicillin, ampicillin, ticarcillin, and piperacillin) and carbapenems (imipenem and meropenem), and it has been suggested its use as a marker for identification of the species (Gordon, *et al*.,2010; Turton(a) *et al*.,2006). The role of ISAba1 and other insertion sequences in modulating the expression of *A. baumannii* resistance genes has also been established for genes involved in cephalosporin resistance (*amp*C) (Turton(a) *et al*.,2006) . *A. baumannii* strains harbor a plethora of plasmids that play key roles in the dissemination of antimicrobial resistance. However, the basic biology of Acinetobacter plasmids is poorly understood. Multiple globally distributed *A. baumannii* strains carry large conjugative plasmids (LCPs) ranging in size from 150 to 200 kb and are characterized by three conserved regions: a locus encoding the type IV secretion system (T4SS) conjugative machinery; a region encoding two TetR transcriptional regulators; and a transposon rich resistance island containing antibiotic resistance genes and regulation the T6SS activity (Di Venanzio(b) *et al*.,2019). Figure 1 showing the Circular map of *A. baumannii*.

pili, also, they do not form spores and flagella are absent (Juni,2015). Morphologically *A.baumannii* is short, plump, typically (1.0–1.5) µm by (1.5–2.5)µm in size as measured during the rapid phase of their growth but often develop into more coccoid in the stationary phase, usually present in pairs or long chains of different in length .*A.baumannii* is metabolically versatile and can be grown easily on simple microbiological media, forming domed, smooth colonies of ˜2 mm diameter, convex, with some species being pigmented pale yellow or grey(Visca, *et al*.,2011). It was able to survive at a low pH(3.37), desiccation, and a high temperature of 40°C (Lal *et al*,2019).

2.4.Epidemiology

Acinetobacter. *baumannii* is primarily a healthcare-associated pathogen and many reports indicated it as the causative of outbreaks and nosocomial infections including septicemia, bacteremia, ventilator-associated pneumonia, wound sepsis, endocarditis, meningitis, and urinary tract infections (Almasaudi,2018; Sen *et al*.,2016). The World Health Organization (WHO) has recently identified antimicrobial resistance as one of the three most important problems facing human health. The most common and serious MDR pathogens have been encompassed within the acronym “ESKAPE,” standing for *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter spp* (Howard *et.al*,2012).

Acinetobacter baumannii became one of the most prevalent pathogens during wars in Lebanon, Afghanistan, and Iraq, causing multiple outbreaks of MDR infections among combat casualties(Bazzi et al.,2020). A key risk group for *A. baumannii* infection is members of the armed forces who have been deployed to conflict zones, particularly Iraq, earning *A.baumannii* the notorious moniker of ‘Iraqibacter.’ The dry, sandy conditions associated with these desert campaigns provide an ideal environment for the physiologically robust *A. baumannii*, making it the main source of infection among injured soldiers(Camp *et al* .,2010).

2.4.1.Habitat:

The natural habitats of the bacteria are soil, water, animals, and humans. It is normal inhabitants of human skin and is frequently isolated from the throat and respiratory tract of hospitalized patients (Fournier(a) *et al*.,2006). However, over the last decade, there have been reports on the existence of *A. baumannii* in environments influenced by humane waste. Urban wastewater represents one of the largest proportions of human waste and it consists of different types of wastewaters that are generated in cities such as domestic, industrial, hospital, and storm wastewaters. Of these, hospital wastewaters are recognized as the source of *A. baumannii* of clinical significance (Higgins *et al*.,2018).

2.4.2. Reasons for outbreaks and methods of transmission

One of the important feature of *A. baumannii* ; its tendency to cause outbreaks due to its resistant antimicrobial agents and its ability to overcome desiccation (Fournier(a) *et al*., 2006). In the past decade strains of *A. baumannii* often have emerged as a major cause of healthcare-associated infections (HAIs)and the organism shows a formidable capacity to complex coexistence of epidemic and endemic infections (Gordon *et al*.,2010). It has the ability to survive on dry surfaces under nutrient limiting conditions facilitates their persistence and transmission in the natural and medical environment. Furthermore, colonized medical devices and equipment could serve as reservoirs in prolonged hospital outbreaks, it is more frequently found on inanimate objects and hands of staff in the (Intensive care unit)ICU than *Staphylococcus aureus* and *Pseudomonas spp*(Almasaudi,2018; Townsend *et al* .,2015).

2.5 Pathogenicity

Acinetobacter baumannii has a wide distribution in most environments and it was considered the second pathogen after *Pseudomonas aeruginosa,* which spread in hospitals (Vishnu Preya *et al*., 2019). The attributable mortalities in patients with *A. baumannii* healthcare-associated infections, of which ventilator-associated pneumonia and bloodstream infections are the most common, can range from 5% in general hospital wards to 54% in the intensive care unit (ICU)(Ayoub Moubareck *et al*.,2020). On the other hand, several various infections are related to this microorganism such as Pneumonia, ventilator-associated pneumonia (VAP) is the most related infection with *A. baumannii* especially in intensive care units (Dexter *et al*.,2015)and bacteremia, It has become a leading cause of bloodstream infections in health care settings with intravenous catheters or the respiratory tract representing a frequent source of infection (Oliveira *et al*.,2015).

Acinetobacter baumannii has been repeatedly isolated from skin and soft tissue in patients with severe burns, wounds, or trauma, for instance, soldiers injured during military operations or victims of natural disasters (Akrami *et al*.,2019; Lerner *et al*.,2020) and it is 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 (Di Venanzio(a) *et al*.,2019). Nosocomial meningitis due to *A. baumannii* remains an increasing threat in intensive care neurosurgery units, with mortality approaching 70%, especially in patients on indwelling ventriculostomy tubes or cerebrospinal fistulae and receiving post-surgical antimicrobial therapy (Xiao *et al*.,2019). However, *A. baumannii* is also reported from osteomyelitis, infections, dental plaques, chronic and aggressive periodontitis, and polymicrobial bacterial infections (Akrami *et al*.2019).

الخلاصة

الراكدة البومانية واحدة من مسببات الأمراض الانتهازية ذات المقاومة المتعددة للأدوية (MDR) ، ويرجع ذلك خصوصا إلى قدرتها العالية على اكتساب المقاومة لمجاميع المضادات الحيوية المتنوعة. تم الحصول على عشرين عزلة (9.7%) من *Acinetobacter baumannii* من (207) عينة سريرية. تضمنت جروح ، حروق ، بلغم ، الدم من الإصابات من كلا الجنسين على حد سواء ، وأعمار مختلفة ، ومناطق محلية متنوعة ، وقد أعطت (162)عينة نمو إيجابي بينما (45) عينة لم يظهر فيها نمو. تم جمع العينات خلال فترة الزمنية من سبتمبر / 2019 وانتهى في نهاية يناير / 2020، من مجموعة من المراجعين والمرضى الراقدين في المستشفيات الحكومية في بعقوبة / ديالى. حيث بلغت نسبة المراجعين (57.97%) بينما بلغت نسبة المرضى الراقدين (42.03%).

التشخيص الأولي لـ *A. baumannii* تم باستخدام الاوساط الزرعية اكار الدم والماكونكي ، واعتمادًا على الميزات على الأوساط الزرعية والاختبارات البيوكيميائية ونظام VITEK 2.وقد تم التأكيد النهائي باستخدام طريقة التشخيص الجزيئي باستخدام تقنية تفاعل البلمرة المتسلسل بواسطة جين bla_{OXA51} للكشف عن نوع A. baumannii. كان العدد الموجب لعزلات *A. baumannii* مرتفعًا في الحروق 8 (40%) ، ثم الدم 7 (35%) ، الجروح 4 (20%) والاخيرة كانت البلغم 1 (5%) عزلة.

تم تحديد الحساسية لمضادات الميكروبات لعزلات *A. baumannii* بطريقة الانتشار القرصي ،لـ 13 مضاد حيوي ، وكانت نتائج مقاومة العزلات كما يلي: (Pipercillin-tazobactam 100%)،(Cefotaxime 100%)، (Ticarcillin-clavulanic acid 100%) ،(أميكاسين 100%) ، (جنتاميسين 100%)،(ليفوفلوكساسين 100%) ،(إيميبينيم 95%)، (ميروبينيم 95%) ، (سيفتريكسون 90%) ،(سيفتازيديم 90%) ،(تتراسيكلين 65%) ،(أمبيسلين - سولباكتام 55%) ، (دوكسيسيكلين 20%).

تمايزت العشرين عزلة من *A. baumannii* قيد الدراسة لنمطين حسب مقاومة المضادات الحيوية في اختبار الحساسية ، جميع العزلات كانت متعددة المقاومة ، 15 (75%) عزلة MDR قاومت (5-9) مضاد حيوي ، 2 (10%) من العزلات XDR قاومت (12) مضاد حيوي ، وثلاث عزلات (15%) من عزلات XDR قاومت كل المضادات الحيوية التي تم استخدامها في الدراسة.

2.6 Genetic Content of *Acinetobacter baumannii*

The chromosome of this bacteria is described as a single chromosome containing 3,976,747 base pairs, 3,454 bp of which are specialized for protein synthesis, and with a DNA G + C content of 39% to 47%, and it has 3830 open reading frame (ORFs). A significant fraction of (ORFs) (17.2%) is located in 28 putative alien islands, indicating that the genome has acquired a large amount of foreign DN (Smith *et al.*, 2007). "A total of 3539 *A. baumannii* genome sequences are available in the NCBI database. All these genomes display resistance traits acquired through horizontal gene transfer (HGT) as part of the accessory genome"(Yakkala *et al.*,2019). It has much strain such as SD002, SDF, and others, but the famous strain is AYE that it as an XDR strain of *A. baumannii* complex.AYE has 86 Kb resistance island (RI) was identified carrying 45 resistance genes (Yakkala *et al.*,2019), and the strain AYE also contains two plasmids, pACICU1 and pACICU2 their size 28.2 and 64.3 base pairs, respectively. The pACICU1 plasmid contains no antibiotic-resistance genes, while the pACICU2 plasmid carries two copies of the *bla*$_{\mathrm{OXA58}}$ gene encoded for Carbapenem resistance. Also, it contains an area of about 20 kbp comprehensive genes involved in transport genes, indicating that this plasmid may be conjugated (Imperi *et al.*, 2011). Most of the *A. baumannii* strains contain on an average 30–40 Genomic islands per genome, indicating a high degree of horizontal mobility of DNA among the strains of the *A. baumannii* complex (Yakkala *et al.*,2019).

The bacteria possesses conserved regions of seven housekeeping gene, and 305- to 513-bp internal fragments of seven such genes—*gltA, gyrB, gdhB, recA, cpn60, gpi*, and *rpoD*—were sequenced for all strains. This housekeeping genes are encoding for this respective products: citrate synthase (*gltA*), DNA gyrase subunit B (*gyrB*), glucose dehydrogenase B (*gdhB*), homologous recombination factor (*recA*), 60-kDa chaperonin (*cpn60*),