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Antibiotic Resistance Patternsand Molecular Typing of*Acinetobacterbaumannii* Clinical Isolates and Hospital Environmentat Alia GovernmentalHospital, Palestine

DoniaMohammad Mosbah Khderat

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B.Sc. in Biologyand Medical Technology / Al-Quds University / Palestine.

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Declaration

I certify that this thesis is submitted for the degree of master is my own research, except where otherwise acknowledged, and that this thesis has not been submitted for a higher degree to any other university or institution.

Signed: .....

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Date: 28 / 5 / 2024.

Dedication

I dedicate this achievement to my family, I would not have reached here without their continuous support and faith in me.

Also, I dedicated this achievement to my supervisor, teachers, friends, colleagues, and everyone who helped me while working on this research.

Last but not least, I dedicate this work to every ambitious soul that turns pain into hope to achieve its dream.

Donia Mohammad Mosbah Khderat

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Antibiotic Resistance Patterns and Molecular Typing of *Acinetobacter baumannii* Clinical Isolates and Hospital Environment at Alia Governmental Hospital, Palestine.

Prepared by: Donia Mohammad mosbah Khderat.

English abstract

Background: In recent years, *Acinetobacter baumannii*received increasing attention;due to theircabability of acquiring multi-resistance to antibiotics and their potential to cause disease in humans. It is also known for its intrinsic antimicrobial resistance mechanisms: several studies documentedthe increase in the percentage of resistance to antimicrobial agents in four majorantimicrobialclasses: Fluoroquinolones,aminoglycosides, β-lactams including Carbapenems. The increase in Carbapenems resistance is of important concern, as thisclass of antibiotics isusually considered the last choice in treating resistant *A.baumannii*.This opportunistic pathogen able to survive in the environment under unfavorable conditions for prolonged periods,this contributes to its increased virulance. Thus, the hospital environment may serve asa reservoir for the resistant*A. baumannii* strainsallowing easily spread in hospital,serve as a nosocomial infections in patients, healthcare personnel, and visitors.

Aims: This study aimed at characterization of *A. baumannii*isolates from inpatients and hospital environments.To find out their antimicrobial resistance pattern, detect Carbapenems resistant *A.baumannii*(CRAB), and to find out genetic relatedness between isolates using random amplification of polymorphic DNA (RAPD) typing technique.

Methodology: In this study, a total of 24 clinical isolates obtained from various clinical specimenssuch as wounds, blood, urine, earswabs and sputumduring the period April1, 2023, and December 31, 2023fromAlia Governmental Hospital, Hebron,Palestine. The Vitek2 system was used for identification and antibiotic susceptibility testing. Concurrently a total of 126 specimen were collected from different environments of the hospital identified as *A. baumannii*using conventional identification methodsand CHROMagar *Acinetobacter*selective medium. The isolates then tested for antibiotic susceptibility testing using disk diffusion methods.All isolates (both clinical and

environmental) were tested for the presence of*bla*$_{\text{OXA-51}}$ gene using PCR. And finally, the genetic relatedness between isolates was determined using RAPDtyping technique.

Results: Twenty fourclinical isolates and 29 environmental isolates were identified as *A. baumannii*. All isolates were confirmed to be *A. baumannii*by detecting *bla*OXA-51-like gene.Among the environmental isolates patient's beds were the most contaminated site (20.6%, 6/29),while the largest number of clinical isolates were obtained from wounds (10 isolates: 41.7%). The majority of clinical and environmental isolates were collected from intensive care unit (ICU).Multidrug resistant *A.baumannii (*MDRAB*)* was detected in approximately 74% of clinical isolates and in 100% of environmental isolates.Co-trimoxazole (COT) was the most effective antibiotic against both clinical and environmental*A.baumannii* isolates. For other antibiotics, clinical and environmental*A.baumannii*isolates showed high resistance rates:86.2% and 96.6% of environmentalisolates were resistant to imipenem (IM) and meropenem (MEM) respectively,while clinical isolates had a resistant rate of 72.7% and 77.3% to IM and MEM, respectively.Using the RAPD-PCR typing technique, four clusters grouped A-D had more than one isolateand the remaining isolates each had unique pattern. Some RAPD clusters were seen with environmental as well as clinical isolates.

Conclusion and recomendations: Thisstudy clearly suggests that the rate of MDRAB and CRAB are increasing and considered an important cause of nosocomial infections.The relatednessbetween clinical and environmental isolates and the detection of high rate of antimicrobial-resistant *A.baumannii*in various hospital environments suggested the potential role of the hospital environments through various clinical activities in the cross contamination of *A.baumannii*infections, particularly in the ICU. These findings highlight the importance of identifying *A.baumannii* infections early and implementing very strict infection control strategies.It is recommended that hospital wards must be vigorously sanitized and disinfected periodically.

Keywords: *Acinetobacter baumannii*, Multidrug-resistant, RAPD typing, Carbapenem resistant *Acinetobacter baumannii* (CRAB), Colistin, nosocomial infections.

نمط مقاومة المضادات الحيوية لبكتيريا الراكدة البومانية المعزولة من العينات السريرية والبيئية من مستشفى عالية الحكومي، فلسطين.

إعداد: دنيا محمد مصباح خضيرات.

إشراف: د. حاتم خالد عيدة.

ملخص

خلفية الدراسة: تلقت الراكدة البومانية في السنوات الأخيرة اهتماما كبيرا بسبب مقدرتها على اكتساب المقاومة للعديد من المضادات الحيوية بسهولة والتسبب في مرض الانسان. كما وتعرف هذه البكتيريا بامتلاكها اليات مقاومة طبيعية، حيث اثبتت العديد من الدراسات زيادة في نسبة مقاومة هذه البكتيريا لكل العوامل المضادة للبكتيريا في اربع فئات رئيسية من المضادات الحيوية :الفلوروكينولونات، الأمينوغليكوزيدات، بيتا–لاكتام بما فيها الكاربابينيم. تعتبر الزيادة في مقاومة الكاربابينيم مصدر قلق كبير، حيث تعتبر هذه الفئة من مضادات الميكروبات عادة الخيار الأخير لعلاج الراكدة البومانية المقاومة للمضادات الحيوية. كما ان هذه البكتريا الانتهازية قادرة على البقاء حية في البيئة تحت ظروف صعبةلفترات طويلة مما يساهم في زيادة انتشار العدوى. وبالتالي ، قد تصبح بيئة المستشفىمصدرا لسلالاتالراكدة البومانية المقاومة للمضادات الحيوية التي تنتقل فيما بعد بين المرضى ,موظفي الرعاية الصحية والزوار.

هدف الدراسة: كان هدفالدراسة توصيف عزلات الراكدة البومانية المأخوذة من المرضى المقيمين وبيئات مستشفى عالية الحكومي، معرفة نمط مقاومتها للمضادات الحيوية،والكشفعن العزلات المقاومة للكاربابينم باستخدام طريقة انتشار القرص ونظام Vitek2،كما وهدفت هذه الدراسة لمعرفة العلاقة الوراثية بين العزلات باستخدام تقنية التضخيم العشوائي للحمض الرايبوزي منقوص الاكسجين(DNA).

منهجية البحث:لاجراء هذه الدراسة، تم جمع24عزلة بكتيرية من عينات سريرية مختلفة مثل الجروح ، الدم، البول، مسحات الاذن، البلغم في الفترة الزمنية الواقعة بين 1/نيسان/2023 و 31/كانون الاول/2023 من مستشفى عالية الحكومي،الخليل، فلسطين. ثم تم تعريفها على انها الراكدة البومانية وتحديد نمط المقاومة للمضادات

الحيويةباستخدامVitek2، اضافة الى ذلك تم جمع 127 مسحة من بيئات المستشفى المختلفةثم تم تعريفها على انها بكتيريا الراكدة البومانيةباستخدام طرق التشخيص التقليدية و الكروم اجار كمستنبت انتقائي لبكتيريا الراكدة البومانية.ثم تم إجراء اختبار الحساسية لمضادات الميكروبات باستخدام اختبار انتشار القرص. باستخدام تقنية تفاعل البوليمريز المتسلسل تم فحص وجود جينbla_{OXA-51}في كل العزلات السرسرسة وغير السريرية. وأخيرا تم تحديد العلاقة الوراثية بين العزلات باستخدام تقنية التضخيم العشوائي للحمض الرايبوزي منقوص الاكسجين(الدنا).

النتائج: تم عزل بكتيريا الراكدة البومانية من24 عينة سريرية و29 مسحة تم جمعها من بيئات المستشفى المختلفة. تم تاكيد جميع العزلات السريرية وغير السريرية على انها بكتيريا الراكدة البومانية عن طريق كشف وجود جين bla_{OXA-51} في كل العزلات التي تضمنتها هذه الدراسة.حيث كانت أسرة المرضى أكثر الأماكن تلوثا (20.6%،29/6). في حين ان غاالبية العزلات السريرية تم الحصول عليها منعينات الجروح (41.7%،26/10). اضافة الى ذلك،فإنغالبية العزلاتالسريرية وغير السريريةتم الحصول عليها من قسم العناية المكثفة. كما أن 73.9% من العزلات السرسرية وجميع العزلات غير السريرية (100%) تبين انها تمتلك خاصية المقاومة للأدوية المتعددة. كوتريموكسازول كان العلاج الأكثر فعالية ضد جميعالعزلات السريرية وغير السريرية.كما وأظهرت العزلات السرسرية وغير السريرية نسب مقاومة عالية للمضادات الحيوية الأخرى التي استخدمت في هذه الدراسة، حيث أن86.2% و 96.6% من العزلات غير السريرية أظهرت مقاومتها للاميبينيم والميوربينم على الترتيب ، بينما أظهرت العزلات السرسرية نسبة مقاومة 72.7% و 77.3% للاميبينيم والميروبينم على الترتيب.باستخدام تقنية التضخيم العشوائي للحمض الرايبوزي منقوص الاكسجين(الدنا)، تم تصنيف العزلات الى 4 مجموعات A–D, كل منها يضم اكثر من عزلة بكتيرية واحدة، بعضها وجد انها تحتوي على عزلات سريرية واخرى غير سريرية، بينما كان لكل عزلة من العزلات المتبقية نمط فريد ومختلف.

الخلاصة:توضح نتائج هذه الدراسة ان هناك تزايد في معدل بكتيريا الراكدة البومانية المقاومة للمضادات الحيوية خاصة في قسم العناية المركزة والتي تعتبر مصدرمهم للعدوى المرتبطة بالمرافق الصحية. كما وأشار التشابه بين العزلات السريرية وغير السريرية لبكتيريا الراكدة البومانية ووجود هذه البكتيريا المقاومة للمضادات الحيوية في

بيئاتالمستشفى المختلفة إلى احتمال مساهمةبيئات المستشفى في نقلالعدوى الناتجة عن هذه البكتيريا من خلال النشاطات السريرية المختلفة. كما وتسلط هذه النتائج الضوء على أهمية الكشف المبكر للعدوىوتطبيق اجراءات الوقاية والمكافحة لمنع هذه البكتيريا من الانتشار.

الكلمات الدالة: بكتيريا الراكدة البومانية، مقاومة العديد منالمضادات الحيوية، التضخيم العشوائي للحمض النووي الريبوزي منقوص الاكسجين(DNA)،بكتيريا الراكدة البومانية المقاومة للكاربابينيم، كوليستين، عدوى المستشفيات.

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List of Abbreviations

Abbreviation	**Term**
A. baumannii	*Acinetobacter baumannii*
AMR	Antimicrobial resistance
WHO	.World Health Organization
ARG_S	Antibiotic resistance genes
HAI_S	Healthcare-associated infections
CRAB	Carbapenem resistant*A. baumannii*
HCW_S	Healthcare workers
RAPD	Randomly Amplified Plyomorphic DNA
CDC	Centers For Disease Control and Prevention.
MDR	Multidrug-resistant
MDRAB	Multidrug-resistant*A. baumannii*
TICU	Trauma intensive care unit
PCR	Polymerase chain reaction

BHI broth	Brain Heart Infusion broth
AST	Antimicrobial susceptibility test
C. isolates	Clinical isolates
E. isolates	Environmental isolates
TEA	Tris glacial Acetc Acid EDTA buffer
CLSI	Clinical and Laboratory Standardes institute
MEM	Meropenem
IMP	Imepenem
COT	Co-trimoxazole
GEN	Gentamycin
AK	Amikacin
CTR	Ceftriaxone
CAZ	Ceftazidime
CL	Colistin
CIP	Ciprofloxacin
MHA	Muller Hinton agar

Chapter One

1.1 Introduction

Antibiotics are drugs commonly employed for the management of bacterial infections in animals and humans, either by killing or inhibiting bacterial growth [1, 2]. They have become an important component of healthcare since they were developed during the 70 years and have made it possible to treat bacterial infections. However, the positive effects of antibiotics on health are greatly challenged due to rising antimicrobial resistance (AMR) levels [3].

One of the major problems facing the world in the twenty-first century is the spread of AMR [4]. It has become a major threat to public health. According to the World Health Organization (WHO) estimates by 2050, AMR will kill 10 million people yearly [5,6]. Bacterial strains may already be innately resistant to a particular antibiotic or they may acquire resistance determinants by integrons, transposons, and plasmids through time [4,7].

In addition, antibiotic resistance is connected with a high risk of morbidity, mortality, elevated treatment costs, and long hospitalization periods [8]. Even though there are numerous causes for antibiotic resistance, it is thought that AMR is an old phenomenon with an accelerated evolution brought on by the misuse and excessive use of antibiotics. Reducing risks is a challenging goal to accomplish because of the great mobility of antibiotic resistance genes (ARGs) and their barrierless diffusion from humans to animals and clinical to environmental reservoirs and vice versa [4].

Recently, in 2020 the WHO presented a list with the names of pathogens for which new antibiotics should be developed urgently; because they developed resistance to most of the antibiotics via the acquisition of ARGsresulting in global health problems, the list includes the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species*) [9].

Acinetobacter baumannii (A. baumannii) is a gram-negative coccobacillus, non-fermenter, non-motile, non-sporulated, oxidase-negative bacterium that is widely

distributed in nature and belongs to the Moraxellaceae family[4]. Species of Acinetobacter mainly *A. baumannii*have become as one of the most significant pathogens in clinical settings.It is considered as an important source of nosocomial as well ascommunity-acquired infections and results in 2 to 10% of the infections caused by gram-negative bacteria [10].

A. baumannii possesses a unique virulence factors that offer benefits at various stages of pathogenesis, beginning from surviving under the immune response of the host toattachment, internalization, and host cell apoptosis. OmpA is one example of an outer membrane protein (OMP) that aids in attachment and internalization into epithelial cells of the host, furthermore it triggers the secretion of apoptotic factors in the host cell, which starts the process of apoptosis and ultimately results in cell death. A.baumannii's capsular exopolysaccharides protects the pathogen from host- and environment-mediated stresses, and their composition dictates the virulence degree. Similarly, A.baumannii specific metal ion uptake mechanism known as nutritional immunity to counteract metal ion chelation mediated by the host. In addition to these, multiple classes of efflux pumps (apart from their actual role in multidrug efflux) also participate in motility and attachment[11].

In recent years *A. baumannii*received increasing attention; because they are easily capable of acquiring multi-resistance to antibiotics (mainly by decreasing the influx and increasing the efflux of the antibiotics, also by modifying the target site and by enzymatic degradation of the antibiotics [12]) and their potential to cause serious infections, as Ventilator-associated pneumonia, Bacteremia (which is commonly results from intravascular and respiratory tract catheter, less frequently, arises from burns, surgical wounds, or urinary tract infections and rarely from endocarditis. A. baumannii blood stream infections overall death rate the ICU ranged from 34.0% to 43.4% and 16.3% outside the ICU),skin/soft tissue infections(as it results in 2.1% of it in ICU).Urinary tract infection (it infrequently cause of UTI and it is responsible for only 1.6% UTIs in ICU and usually linked to catheter use), Meningitis (it caused approximately 10% of Gram-negative bacillary and 4% of all nosocomial meningitides), in addition to the previous infections few studies reported *A. baumannii* endocarditis that commonly

included prosthetic valves. endocarditis, peritonitis, ophthalmitis or keratitis may cause by *A.baumannii* as a result of using contact lens use or following eye surgery[4,12, 13,14].

The phenotypic methods andbiochemical profile of *Acinetobacter species*was inadequate for correctly recognizing and distinguishing the highly related Acinetobacter species. Later, molecular methods, especially DNA-DNA hybridization, have been used to identify at least 33 different *Acinetobacter genospecies*. Genospecies 2, *A.baumannii*is the most clinically significant species and accounts for about 90% of clinical infections caused by *Acinetobacter spp*. Therefore,it is considered an important health-care-associated pathogen [15, 16].

In hospital settings, *A. baumannii* is well known for its capacity to colonize and/or infect critically ill people. Invasive surgeries and instruments, lengthy hospital stays, wide-spectrum antibiotic use, and the severity of underlying illness are all risk factors for *A. baumannii*colonization and infection[17].

In addition, *A. baumannii* is known for its intrinsic antimicrobial resistance mechanisms. Several studies proved an increase in the percentage of its resistance to all antimicrobial agents in four major classes of antimicrobials: Fluoroquinolones, aminoglycosides, β-lactams including Carbapenems [16]. Carbapenem resistant *A. baumannii* (CARB) poses a severe hazard to hospitalized patients as it results in about 8500 infections of the patients in hospital and 700 deaths in the USA[18]. Therefore, the increase in carbapenem resistance is of important concern, as this type of antimicrobial is usually considered the last choice of defense in treating resistant *A. baumannii.* As a result, the WHO regards CRAB as its top critical priority pathogen, for which new therapies are urgently required and there are growing worries that hospital-acquired *A. baumannii* infections won't be treatable soon if there isn't a big intervention[19].

Moreover, this opportunistic pathogen has the potential to survive in the environment under unfavorable conditions for prolonged periods which may contribute to its increased infections. Thus, the environment may become a reservoir for the resistant*A. baumannii*strains that easily spread in hospitals and may also be a source of nosocomial infection for patients, healthcare personnel, and visitors[19].

Several studies have considered *A. baumannii*as a dangerous nosocomial. Although *A. baumannii*infections acquired in hospitals are being reported more frequently worldwide, the similarities between *A. baumannii*isolates from various medical settings and patients, however, have received little attention.

In Palestine, Alagha, H. et al. in 2021 reviewed most published papers on bacterial antibiotic resistance of clinical isolates as well as isolates from hospital environments in the Gaza Strip/Palestine over 20 years. The results of these investigations have shown elevated rates of multidrug-resistant gram-negative bacteria, especially *E. coli*, *Enterobacter spp*, *Klebsiellaspp*, *Acinetobacter spp*, and *Pseudomonas aeruginosa* throughout the study [20].

Another study carried out in a COVID-19 dedicated hospital (Academic HasharonHospital) in April 2019 described an outbreak of CRAB in the hospital and thought that the healthcare workers' ($HCW_{S)}$hands or equipment and environmental sources which continued even after the hospital was evacuated and thoroughly cleaned were the source of infection[21].

Knowing antibiotic resistance pattern of *A.baumannii* is essential for applying infection control plans, minimizing antibiotic use while maximizing it's efficacy, and subsequently improving patient prognosis. Furthermore, the incorporation of environmental samples into this study offered a distinct perspective to our knowledge of the sources and patterns of transmission of antibiotic-resistant bacteria in hospitals.

To date, there have been few studieswhich have investigated antibiotic resistance pattern, epidemiology, and hospital environment role in the transmission of *A.baumannii* in Palestine. therefore, this study aimed to characterize and determine the extent of antimicrobial resistance pattern of *A. baumannii* isolates from inpatients and hospital environment, to detect the CRAB. Moreover, to find out the genetic relatedness between these isolates using RAPDtyping technique.

1.2 Literature review

The Centers for Disease Control and Prevention (CDC) identified multidrug-resistant (MDR) *Acinetobacter* as one of 12 'serious' public health threats: infection could worsen and become a real threat if not monitored. In its first-ever report on antibiotic resistance in 2013. In a newly released 2019 update to this report, CRAB was raised to the threat level of 'urgent' because of the emergence of easily spread resistance and the lack of current antibiotics or new antibiotics in development to treat CRAB infections [22].

Roberts, S. et al. (2001) examined how the multi-drug resistant*A. baumannii (MDRAB)* spreads during an outbreak in the burns intensive care unit. They discovered that the environment in the burns room was contaminated with *A. baumannii*, as the handle of the door that leads from the antechamber between the two rooms, which allowed HCWs' hands to become contaminated. They also discovered that all except one of the environmental isolates and the isolates from patients and HCWs shared the same genetic pattern [23].

In 2003, BreeanBeggs's research addressed "The Airborne Transmission of Infection in Hospital Buildings: Fact or Fiction?" [24], this study concluded that although spread by contact is the primary method of transmission for the majority of infections, the contribution of airborne organisms to the infection spread is more than is recently known. This is due in part to the fact that many airborne organisms are viable but unculturable, making them difficult to detect, and also because some infections resulting from contact transmission involve the airborne transportation of microorganisms onto inanimate surfaces [24].

A study was carried out by Scott, P. et al. [25] to evaluate patients' skin, soil, and hospital environments as potential sources of the outbreak of multidrug-resistant *Acinetobacter baumannii-calcoaceticus*complex in the health care system related military operations. They found out that the clinical isolates were less drug-susceptible than environmental isolates, and the hospitals with environmental contamination as well as the spread of infections within healthcare facilities were key contributors to this outbreak [25].

Another study, investigated the antimicrobial susceptibility and genetic relatedness of environmental and clinical isolates during the outbreak of *A. baumannii*in a neonatal ICU and showed that *A. baumannii*isolatesare resistantto all β-lactams and resistant other antibiotics with different degrees but all isolates were sensitive to colistin and outbreak isolates analysis indicatethat the isolates are genetically identical to the outbreak strain [26].

In another study, Cicek, A. et al. analyze clinical and environmental isolates of *A. baumannii* to look into the origin of the outbreak affecting ICU patients. All of the isolates exhibited multidrug resistance, including resistance to the antibiotic classes of Cephalosporins, Carbapenems, Fluoroquinolones, and Aminoglycosides. According to this study, all *A. baumannii*isolates had a common source and the patient's environment was the source of the outbreak [27].

The MDRAB outbreak that occurred in an internal medicine unit was studied, including its clinical, microbiological, epidemiological, and molecular characteristics. The results showed that the environmental samples belonged to the same clone and thus had a common infection source that is likely the HCW_S' hands; because the outbreak strain was discovered on the serum container surface [17].

In 2013, the clonality of both air and clinical isolates and the presence of *A. baumannii*contamination in the Trauma ICU's ventilation system and air were studied, the results of this study discovered that *A. baumannii*was present in the air and air ducts, raising concerns about aerosolization of *A. baumannii*in ICU and calling for more research into its role in *A. baumannii*transmission between patients. It has been found that all air isolates were resistant to Carbapenem and related to clinical isolates of patients present in the TICU [28].

*A. baumannii*isolates from hospital environments and respiratory tract samples of patients hospitalized in adult intensive care units were examined for main biological features. The study indicates that all MDRAB isolates from hospital patients' respiratory tracts and the environment contained remarkably identical multidrug resistance patterns and biological traits. *A. baumannii*strains remain in the hospital setting, particularly in water and wet

conditions, and they generate biofilm, which may be the cause of the high colonization rates in the ICU patients' respiratory tracts[29].

In 2014, Rosa, R. et al. conducted research on *A. baumannii*-negative patients to study the relationship between environmental exposure to CRAB and the risk of subsequently contracting this pathogen. They conclude that, those who were exposed to a contaminated hospital environment had a greater probability of contractingCRABcompared to patients who were not exposed[30].

Risk factors for acquiring the MDRABduring an outbreak in a burn unit were looked into (by Simor, A. et al. in 2014). It has been found out that these risk factors were likely multifactorial and related to environmental contamination and contact with intermittently colonized HCW_S[31].

Drug resistance, genes encoding OXA-type carbapenemase, and genetic variation in airborne *A. baumannii* were studied by Gao, j. et al. and it has been found that airborne *A. baumannii*exhibited complex molecular diversity, multidrug resistance, and major carbapenem resistance mechanisms in the form of OXA-23 and OXA-51 [32].

In 2016, Aliramezani,A. et al. conducted astudy to evaluate the prevalence of *CRAB i*n the hospital setting and to describe their clonality, susceptibility profile, blaOXA gene carriage, and biofilm development, this study proved the presence of clonally-related OXA-23-producing *CRAB* in hospital environments in high-risk areas of referral hospitals, as well as the distribution of MDRABon tools frequently used for patient care, such assuction tubes or dressing sets, and on surfaces touched by staff and patients, like sinks, which may elevate the risk of transferring resistant isolates to susceptible patients [33].

Kateete, D. et al.(2016) in a study aimedto find out how common Carbapenems-resistant strains of *P. aeruginosa* and *A. baumannii* are and whether the hospital environment is home to Carbapenems-resistant Gram-negative rods. They concluded that there was a significant prevalence of Carbapenems-resistant *P. aeruginosa* and *Acinetobacter* in the environment, which is concerning because the hospital setting could be a source of infection for patients who are at the facility as well as for medical personnel [13].

In a study carried out by Shamsizadeh, Z. et al. in 2017, antibiotic-resistant *A. baumannii*presence in many hospital environments in different wards, air, water, and inanimate surfaces was investigated, the results showed that antibiotic-resistant *A. baumannii*was detected in many samples thus hospital environments may act as a possible source for *A. baumannii*infections transmission specifically in ICUs [34].

In 2017, *A. baumannii*was obtained from a hospitalenvironments to investigate its antibiotic resistance, virulence factors, and resistance gene determinants. The antibiotic susceptibility test showed that all the *A. baumannii*isolates were multidrug-resistant with 100% resistantto gentamycin, ciprofloxacin and 100% sensitive to colistin and tigecycline, also had many virulence factors and long-term resistance to dry conditions whichresults in a dangerous public health problem[35].

In 2017, Raro, O. et al. studied*A. baumannii* presence in the ICU environment and ICU worker's gloves and compared the antimicrobial resistance of the isolates with isolates from ICU patients at the same hospital. It was found a high prevalence of MDR and the antimicrobial susceptibility profiles of environmental and clinical *A. baumannii*isolates were strongly similar, as well as all isolates that were resistant to Carbapenems had bla OXA-23. These findings point to the ICU environment as a potential source for MDRABpersistence, and as a result, the ICU may be a source of nosocomial infections results by this microbe [36].

In another study carried out by Janbakhsh, A. et al. (2020) aimed to determine the antibiotic resistance pattern of *A. baumannii*in ICU units. It was found that isolates of *A. baumannii*from patients, healthcare workers, and ICU instruments were highly resistant to many antibiotics and were 100% resistant to cefepime, ciprofloxacin, ampicillin, ceftriaxone, ceftazidime, cefazolin, trimethoprim, gentamycin, piperacillin, and imipenem [37].

The role of hospital the environment in the transmission of CRABin a burn intensive care unit was studied by Shenoy, E.et al. (2020), the results indicated that the isolates were resistant to various antibiotics to which resistance is unpopular at the studied hospital toampicillin-sulbactam, gentamicin, and trimethoprim-sulfamethoxazole, also it revealed that environmental sampling identified heavily contaminated areas and isolates from

which highly identical to the clinical isolates. Therefore, determined the role of environmental reservoirs in the infection transmission [38].

In a recent study, Bakhshi, F. et al. (2022) examined *A. baumannii*isolates from burn patients and the hospital environment for antibiotic susceptibility and the presence of the most prevalent OXA-type carbapenemase genes, the findings indicated that surfaces in the hospital environment, especially in ICUs are contaminated with MDR or XDR *A. baumannii* strains and may be thought of as a possible reservoir for the hospital patients. Additionally, one of the primary mechanisms of Carbapenems-resistance in the clinical and environmental *A. baumannii*strains appears to be OXA-type carbapenemases, including OXA-23-like and OXA-24/40-like [39].

Additionally, Shali, A. et al. (2022) did a study to detect and analyze *A. baumannii*isolates from inpatients and hospital environments, the results revealed that resistance was greater in clinical isolates than in environmental isolates and a high prevalence of MDRABwere shown, it was found also that hospital patients' and the environmental isolates had similar genotypes during the study period, suggesting infection transmission route within the hospital [40].

1.3 Problem statement and Justification

Currently, one of the biggest risks to public health is antibiotic resistance[41]. Around the world, resistance rates are continuously rising, with 40–70% of infections being resistant to carbapenems[42]. Which raises mortality, morbidity, and treatment costs in addition to limiting the options for therapy.

*A.baumannii*is an opportunistic pathogen that is frequently linked to aquatic environments. It produces serious and aggressive nosocomial and community-acquired infections [43]; because of its ability to acquire resistance to most antibiotics resulting in its ability to survive for months under harsh and dry conditions that make it difficult to treat and eradicate which makes it of great concern[44]and resulting in MDR strains of *A. baumannii* that is occurring more common in hospitals and is now considered an urgent global health issue [45].

Therefore, the establishment of successful strategies for infection control and prevention must overcome by the lack of in-depth knowledge of the patterns of antibiotic resistance in clinical and environmentalisolates and the possible environmental reservoirs of *A. baumannii*strains.

1.4 Aims and Objectives

1. To isolate and identify *A. baumannii*from different clinical and environmental samplesobtained from Alia governmental hospital.
2. To determine the antibiotic resistance pattern of environmental and clinical *A. baumannii*isolates
3. To explore theCRAB isolates.
4. To determine the genetic relatedness between isolates using RAPD technique

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Chapter Two

Methodology

2.1 Materials

The materials used in this research are illustrated in table 2.1.

Table 2.1: Materials and instruments used in the study.

No.	Item	Components	Provider
1.	Chrome agar	Agar (15g/L) Peptone and yeast extract(12g/L) Salts(4g/L) Chromogenic mix(1.8g/L) Growth and regulator factors(4mL/L) 5 vials of MDR supplement. - Final pH (at 25°C) 7.0±0.2	CMC company
3.	Antibiotics	- Meropenem (10 µg). - imipenem (10 µg). - Co-trimoxazole (1.25/23.75 µg). - Gentamycin (10 µg). - Amikacin (10 µg). - Ceftriaxone (30 µg). - Ceftazidime (30 µg). - Colistin (10 µg). -Ciprofloxacin (5 µg).	Bioline
4.	Gel electrophoresis procedure	- Agarose powder - Tris EDTA buffer(TAE) 1x - Microwave - Ethidium Bromide solution - DNA ladder 100-1500 bp(Fermentas) - Bio-Rad gel electrophoresis apparatus	Hylabs

		- UV- Light documentation system	
5.	PCR	- PCR tubes - Eschohealthcare Swift PCR thermos- cycler device - PCRBIO HS Taq Red master-mix - Heat block 95°C - Microcentrifuge	Hylabs
6.	Primers	- OXA-51-like forward and reverse F: 5'-TAATGCTTTGATCGGCCTTG-3' R: 5'-TGGATTGCACTTCATCTTGG-3' - RAPD single primers 5′-AAGACGCCGT-3′	Hylabs

2.2 Methods

2.2.1 Bacterial Isolates:

The *A. baumannii* clinical as well as environmental isolates were obtained from various clinical and environmental samples from Alia Governmental Hospital, Hebron, Palestine during the period April1, 2023, and December 31, 2023.

2.2.1.1 Clinical isolates:

A total of 24 clinical isolates obtained from various clinical specimen such as blood, wound, urine, ear swabs, sputum(Table 2.2) fromAlia Governmental Hospital, Hebron,Palestine. The isolates were identified as *A. baumannii* by the Vitek 2 automated system (VITEK®2 ID & AST CARDS-BioMerieux, UK, Ltd.). These isolates were sub-cultured on MacConkey and incubated aerobically at 37°C for 24 hours then frozen at -20°C in Brain Heart Infusion Broth (BHI broth) that contains 40% glycerol for additional analysis[46].

Table 2.2: Clinical A.baumannii isolates fromdifferent sources.

No.	Source	No. of samples
1.	Wound	10
2.	Blood	5
3.	Urine	3
4.	Ear	2
5.	Sputum	4
Total		**24**

2.2.1.2 Environment isolates:

Concurrently with the clinical isolates, a total of 126 samples(Table 2.3) were collected from different environment locations of the hospitalusing saline-moistened sterilized swabs (coded E) such as patients' beds, bedside tables, vital signs measuring devices, rubbish bins, door knobs, sinks, windows, ventilators, suction bottles, chairs, curtains, medication tables, counters, healthcare worker's and cleaner's gloves etc[47]

Out of the 126 environmental sample, 29 were identified as*A. baumannii*.All isolates were incubated in BHI broth overnight at 37°C [36]. Then the isolates were cultured on CHROMagar *Acinetobacter* (CHROMagar, Paris, France) selective mediumand incubated aerobically at 37°C for 24 hours [48].

The isolates were identified to species level using conventional identification methods according to (Man Hwan et al, 2015). Briefly the isolates were sub-cultured on MacConkey's agar plates at 37 °C for 24 h. The *A. baumannii* were identified by colony morphologyand biochemical reactions: catalase, oxidase, OF (Oxidation and fermentation, and API 20NE system (BioMérieux Co, France)[49]. The positive samples for *A. baumannii*were sub-cultured on MacConkey agar, then frozen at -20°C in BHI broth that contains 40% glycerol for additional analysis [46].

Table2.3: EnvironmentalA.baumannii samples from different sources.

No.	Source	No. of samples
1.	Patient's bed	14
2.	Bedside tables.	7
3.	Vital signs measuring devices.	6
4.	Keyboards.	2
5.	Rubbish bin.	6
6.	DoorKnob.	15
7.	Sinks.	15
8.	Windows.	3
9.	Ventilators.	15
10.	Suction bottles.	5
11.	Chairs.	3
12.	Curtains.	2
13.	Medication tables	9
14.	Urine catheter	1
15.	Central line	3
16.	IV stand	4
17.	Gloves	6
18.	Electrical outlet	5
19.	Walls	4
20.	Refrigerator	1
Total		**126**

2.2.2 Molecular identification:

All isolates were identified and confirmed by PCR detection of *bla*$_{\text{OXA-51}}$ gene [50]. The primers used in this study are shownin the Table 2.4 [34, 40, 51, 52]. Vitek2 confirmed *A. baumannii* isolate DNA and DNA-free reaction, were used as positive and negative control, respectively [36].

Table 2.4: Primers sequences used in this study for blaOXA-51 and RAPD.

Primer	Sequence	Reference
blaOXA-51	F: 5'-TAATGCTTTGATCGGCCTTG-3' R: 5'-TGGATTGCACTTCATCTTGG-3	32,38,49,50
RAPD	5′-AAGACGCCGT-3′	2

2.2.2.1 DNA extraction:

The boiling method was used to extract DNA from study isolates. Briefly, 2-3 isolated colonies of each isolate were taken from the overnight culture of *A. baumanni*on MacConkey plates and immersed in 200µL of distilled water then were boiled for 10 minutes at 95°C, then the suspensions were centrifuged for 10 minutes at 13,000 rpm(Biofuge fresco), and finally the supernatant was used as a DNAtemplet [53]. Until use, extracted DNA was stored at -20°C [54].

2.2.2.2 PCR amplifications:

PCR reactions were carried out in 25µl final volume including 12.5µl of 2x master mix, 5.5µl of sterile double distilled water (DDW), 1 µl of each primer (10 µM/µl), and 5µl of DNA templet. Amplifications were performed in the thermocycler (Escohealthcare swift. Max Pro)[50].PCR programare shown in the table 2.5.

In all PCR experiments, negative and positive controls were included.

Table 2.5: PCR programfor blaOXA-51-like gene.

	Step	Temperature	Time
A	Initialization	94°C	3 minutes
B	Denaturation	94°C	30 seconds
C	Annealing	56°C	30 seconds
D	Extension	72°C	30 seconds
Steps from B – D were repeated 34 cycles.			
E	Final extension	72°C	5 minutes

2.2.2.3 Agarose gel electrophoresis:

Briefly, 10µL of amplicons from each mixture of reaction were taken and analyzed using1.5% (w/v) agarose gel in tris glacial Acetic Acid EDTA buffer (TAE) at 110 voltages for 40 minutes and visualized with ethidium bromide staining, then the gels were photographedunder Ultraviolet light.A DNA ladder with a molecular size of 100-1500 bp(Gene DireX, hy-labs)was used to compare the molecular sizes of the PCR products

2.2.3 Antibiotic susceptibility test (AST):

2.2.3.1 AST for clinical isolates:

The antibiotic resistance pattern of all clinical isolateswas determined using the Vitek 2 automated system (VITEK®2 ID & AST CARDS-BioMerieux, UK, Ltd.) atAlia governmental hospitalfor the following antibiotics: Meropenem (MEM, 10 µg), Imipenem (IMP, 10 µg), Co-trimoxazole (COT, 1.25/23.75 µg), Gentamycin (GEN, 10 µg), Amikacin (AK, 10 µg), Ceftriaxone (CTR, 30µg), Ceftazidime (CAZ, 30 µg) and Ciprofloxacin (CIP, 5 µg).

2.2.3.2 AST for environmentalisolates:

Disk diffusion method on Muller Hinton agar (MHA) was used to test the resistance pattern of all environmental isolates using the following antibiotics: Meropenem (MEM, 10 µg), Imipenem (IMP, 10 µg), Co-trimoxazole (COT, 1.25/23.75 µg), Gentamycin (GEN, 10 µg), Amikacin (AK, 10 µg), Ceftriaxone (CTR, 30 µg), Ceftazidime (CAZ, 30 µg), Colistin (CL, 10 µg), and Ciprofloxacin (CIP, 5 µg) as recommended by Clinical and Laboratory Standards (CLSI) [55].For interpretation of colistin susceptibility, ≤8 mm and ≥11 mm were considered as resistant and sensitive, respectively [56].

2.2.4 Molecular typing by RAPD–PCR:

RAPD–PCR was carried out using 10-mer random primer [2] (Table 2.4) for determining genetic relatedness between *A. baumannii*isolates. DNA template from each strain was extracted as explained previously [53]. After that, in a total volume of 25 µl, PCR was performed using thermocycler (Escohealthcare swift. Max Pro) utilizing 12.5 µl 2x master mix, 6.5 µl DDW,1 µl of primer (10 µM/µl),5µl of DNA templet, under the following conditions: Initial denaturation step 15 min at 94°C, then 40 cycles for 1 min at 94°C, for 1 min at 37°C, and 2 min at 72°C as final extension[2]. RAPD-PCR were carried out for all isolates in one PCR run.

Thenagarose gel electrophoresis was performed as mentioned above.A DNA ladder with a molecular size of 100-1500bp was used to compare the molecular sizes of the PCR-RAPD products [7].

Chapter Three

Results

3.1 Bacterial isolates

Atotal of 53bacterial isolateswere collected from Alia governmental hospital/Hebron/West Bank, 24samplesfrom hospital clinical laboratoryand 29isolatescollected from the hospital environments.

3.2 Phenotypic identification of *A. baumannii*isolates

The 24clinical isolates (19 were from males while the remaining from females) identified as *A. baumannii* by VITEK® 2 COMPACT systemand the29 hospital environment isolates.were identified as *Acinetobacter spp* by the growth of red colonies on CHROMagar(Fig.3.1),then the colonies that appeared as gram-negative coccobacillus by gram stain,palepink color, non-lactose fermenter on MacConkey agar (Fig.3.2), catalasepositive, oxidase negative, oxidationpositive and fermentation negative using OF testwere initiallyidentified as *A. baumannii*. Finally the isolates were confirmed as A.baumannii using the API system.

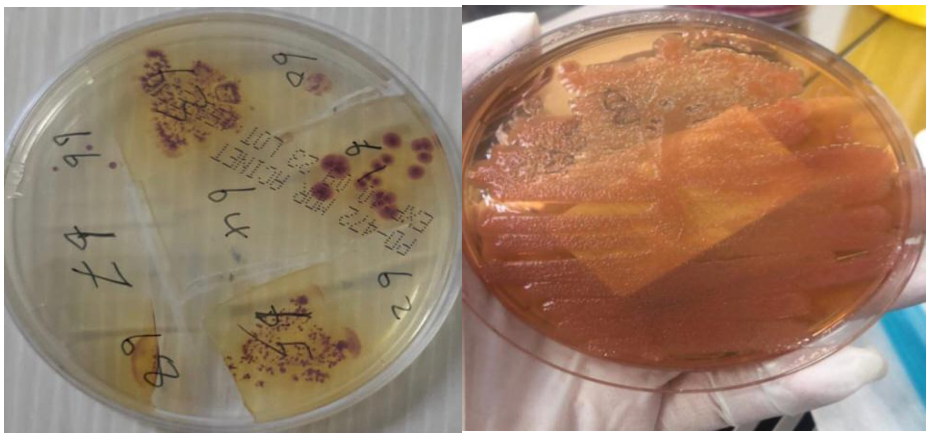

Fig. 3.1*A. baumannii* on CHROMagar.Fig. 3.2: *A. baumannii* on macConkey agar.

3.2 Molecularidentification of *A. baumannii* isolates

Twenty-four of clinical isolates and 29 of environmental isolates were further confirmed as *A. baumannii* by bla OXA $_{51}$ PCR(fig 3.3).

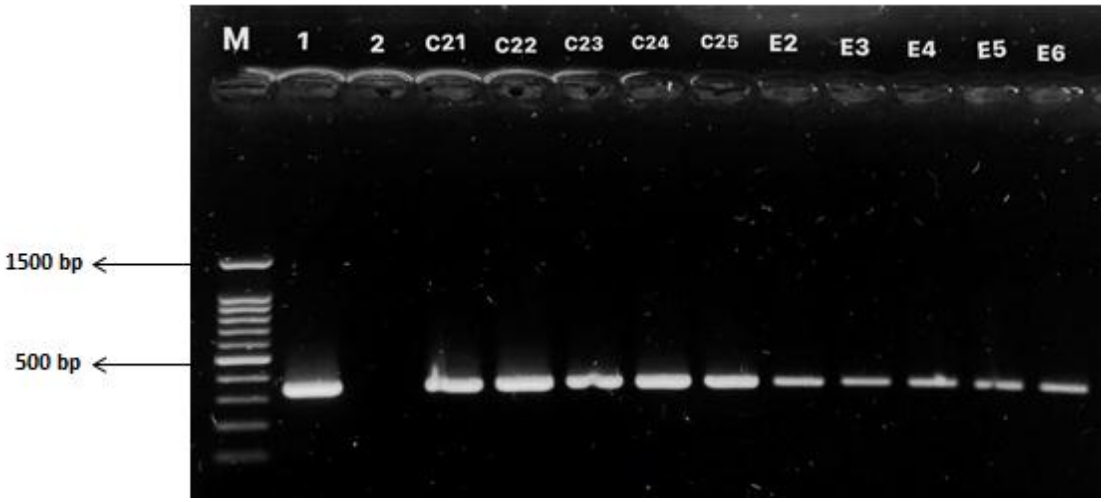

Fig 3.3: Detection of *bla*OXA-51 genes of *A. buamannii*in some clinical and environmental isolates**.**Lane M: 100 bp DNA Ladder, Lane 1: positive control (C_{20}), Lane 2: negative control, LanesC_{21}– $C_{25:}$clinical Isolates and E_2-E_6: Environmental isolateshaving blaOXA-51 band (353 bp).

In our study, most of the clinical isolates were under15 yearsof age followed by age grouop more than60 years as demonestrated in fig.3.5.

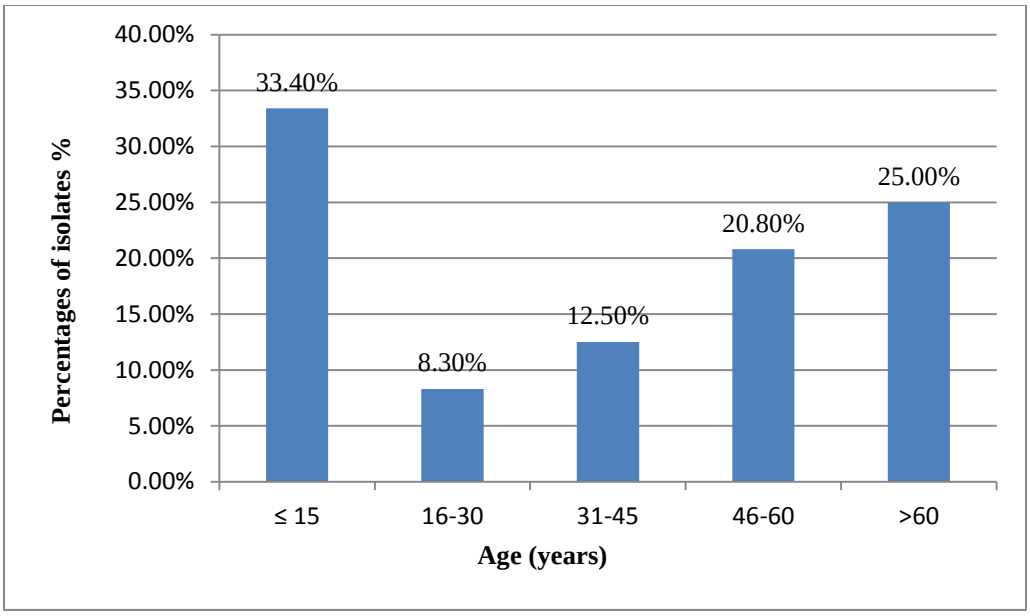

Fig.3.4: Distribution of*clinical A. baumannii* isolates basedon age groups .

The majorityofclinical isolates were obtained from wound (10 isolates: 41.7%) followed by blood (5 isolates: 25%). As indicated in Table 3.1. Whereas the majority of environmental isolates were collected from patient's bed (6 isolates:20.6%) followed by sink (5 isolates:17.2%),Bedside tables and doorknob (4 isolates: 13.8%) as indicated in Table 3.2.

Table 3.1: The percentage of clinical *A. baumannii* isolates from different sources.

Source	Percentage %
Wound	41.7%
Blood	20.8%
Sputum	16.7%
Urine	12.5%
Ear	8.3%

Table 3.2: The percentage of environmental*A. baumannii* isolates from different sources.

Source	Percentage %
Patient's bed	20.6%
Sink	17.2%
Bedside table	13.8%
Door handle	13.8%
Vital signs measuring devices.	10.3%
Oxygen Ventilators.	6.8%
Window	3.5%
Medication table	3.5%
Chair	3.5%
Curtains.	3.5%
Rubbish bin	3.5%

Out of the 24 clinical isolates, 3 isolates(12.5%) were obtained from outpatients and 21 isolates (87.5%) were obtained from inpatientsThe majority of clinical and environmentalisolates were collected from ICU ward(11 isolates:45.8% and 22 isolates:75.9%,respeectively) as shown in table 3.3 and table 3.4.

Table 3.3: The percentage of clinical *A. baumannii* isolates from different hospital wards.

Ward	Percentage %
ICU	45.8%
Pediatric	16.6%
Burns	16.6%
Hemodialysis	4.2%
Internal medicine	4.2%
Surgery	4.2%
Day care	4.2%

Emergncy	4.2%

Table 3.4: The percentage of environmental*A. baumannii* isolates fromdifferent hospital wards.

Ward	Percentage %
ICU	75.9%
Pediatric	10.3%
Obstetrics & Gynecology wards	10.3%
Internal medicine	3.5%

3.3 Antibiotic Susceptibility testing for *A. baumannii* Isolates.

Allclinical as well as environmentalisolates were subjectd to antimicrobial susceptibility disk diffusion testing using the following eight antibiotics:CIP, MEM, IMP, AK, GEN, CTR,CAZ and COT. CL was tested only forenvironmental isolates.

The resistance rates of *A. baumannii* isolates are shownin Fig.3.6. Both groups revealed high resistance rates to t alltestedantibiotics except to COTwhich was the most effective antibiotic with the lowest resistance ratesagainst both clinical and environmental isolates15.4% and 41.4%, respectively.Furthermore, the resistance rates were higher in environmental isolates (which were100% resistance to AK, GEN, IMP, CTR) in comparison toclinical isolates.

Theenvironmentalisolatesshowed high resistancetoIM and MEM with resistance rate of 100% and 96.6%, respectively, while clinical isolates had also high resistant rate, 72.7% and 77.3% to IM and MEM,respectively.Moreover, 72.4% ofthe environmental isolates showed intermediate resistancetocolistin and 20.7% showed complete resistanceas shown in Fig.3.7.

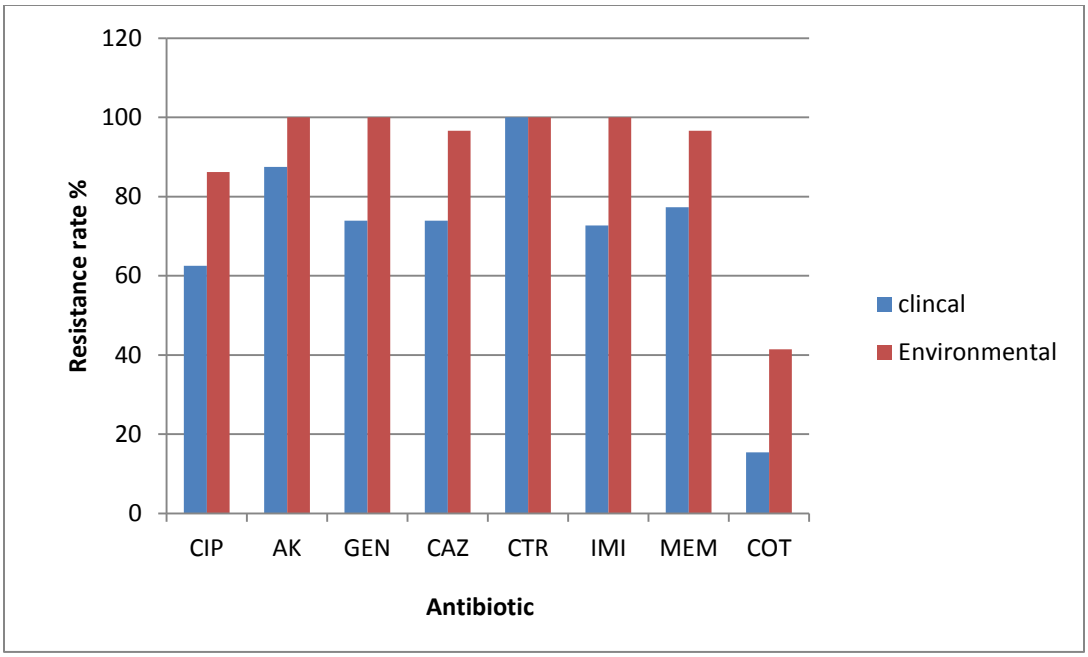

Fig.3.5: Resistance rates of *A. baumannii* isolates to different antibiotics.

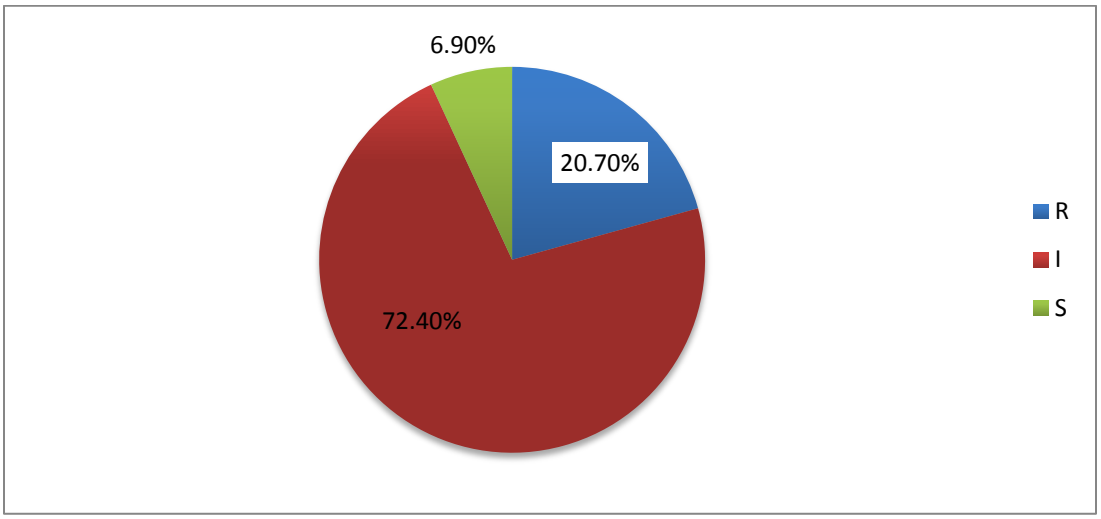

Fig.3.6: Resistance pattern of environmental*A. baumannii* isolates to colistin.

The reported rate of MDR are approximately 74% (17) ofclinical and 100% (29) ofenvironmental*A. baumannii* isolates. revealed intermediate or full resistance to at least one antibiotic in three or more antimicrobial classes.The maximum number of clinical MDR strains were isolated from wound followed by blood while the majority of environmental MDR strains were isolated from patients beds followed by bedside tables .The highestMDRABwere detected in ICU as shown in fig 3.8. The maximum number of

clinical MDAB in the ICU were from blood followed by sputum while the patient's beds followed by bedside tables were the most contaminated sites in the ICUwith MDAB. Furthermore, males had higher MDR isolates(76.5%) in comparasion to females(23.5%).

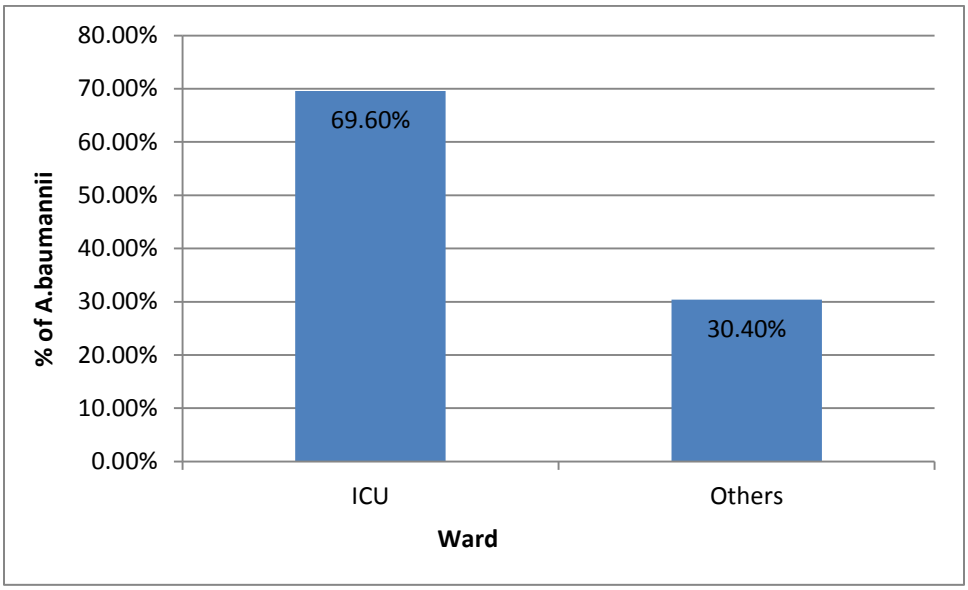

Fig.3.8: The distribution of MDRAB isolates from different hospital wards.

3.4 Molecular typing of *A. baumannii* isolates

Four clusters grouped as A-D were identified by RAPD-PCR (fig.3.9). All isolates with≥ 1 band difference by comparing bands molecular size on agarose gel of each isolate with the molecular size of DNA ladder bands were included in the same cluster.

As shown in table 3.5,theisolates of strain D were mostly from ICU and the remaining were from pediatric, burns, surgery, etc, the majority of which were clinical and the remaining were environmental. Nineteen isolates of strain A were mostly from ICU except one which has been isolated from pediatric ward and all were environmental. Five clinical isolates of strain C were from four different wards of the hospital. Two environmental isolates of strain B were obtained from ICU. The remaining 8 isolates, each had unique pattern.

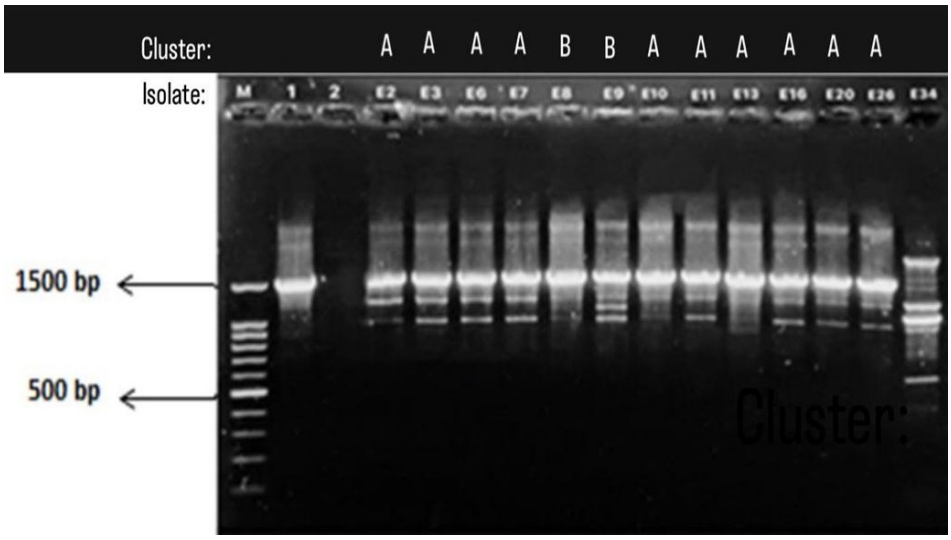

Fig.3.8: RAPD-PCR electrophoresis of *A. baumannii*.

Lane M: 100 bp DNA ladder (100bp – 1500 bp). Lane 1: Positive control (C_{20}). Lane 2: Negative control. lanes E_2 –E_{34}: Some of examined environmental *A. baumannii* strains.

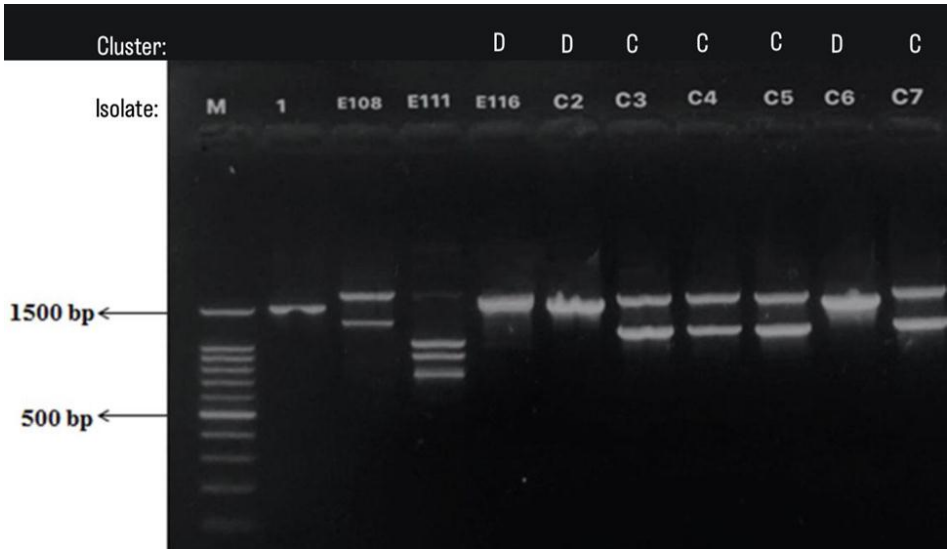

Fig.3.9: RAPD-PCR electrophoresis of *A. baumannii*.

Lane M: 100 bp DNA ladder (100bp – 1500 bp). Lane 1: Positive control (C_{20}). Lanes E_{108} – E_{116}: Some of examined environmental *A. baumannii* strains. lanes C_2 –C_7: Some of examined clinical *A. baumannii* strains.

Table 3.5: The type and the source of isolates of the four clusters of RAPD-PCR.

Cluster	Name of isolates	Ward
*A	E_2, E_3, E_6, E_7, E_{10}, E_{11}, E_{13}, E_{16}, E_{20}, E_{26}, E_{36}, E_{37}, E_{39}, E_{42}, E_{48}, E_{52}, E_{53}, E_{66}	ICU
	E_{70}	Pediatric
**B	E_8, E_9	ICU
***C	C_3, C_4	ICU
	C_5	Internal-Medicine
	C_7	Pediatric
	C_{12}	Burns
****D	C_2, C_9, C_{15}, C_{16}, C_{17}, C_{18}, C_{21}, C_{23}, C_{26}	ICU
	E_{81}, C_{20}, C_{25}	Pediatric
	C_{19}, C_{22}, C_{24}	Burns
	C_6	Surgery
	E_{116}	Obstetrics & Gynecology
	C_{10}	Day care
	C_{14}	Emergncy

*: Isolates of this cluster were from patient's bed, bedside table, vital signs measuring devices, patients bed, door knob, window, door knob, ventilator, medication table, patient's bed, curtains, patient's bed, bedside table, chair, patients bed, vital signs measuring devices,vital signs measuring devices, door knob, patients bed, recpectively.

**: Isolates of this cluster were from bedside table, rabbish bin, recpectively.

***:Isolates of this cluster were from blood, wound, wound,blood, wound, recpectively.

****: Isolates of this cluster were from bedside table, sink, sink, blood, wound, sputum, ear swab, sputum, wound, urine, wound, sputum, blood, blood, wound, urine, blood, wound, wound, urine, recpectively.

Chapter Four

4.1 Discussion

*A. baumannii*has emergedas an important nosocomial pathogento human due to its resistance to most antibiotics and disinfectants, leading to life-threatening nosocomial infections[57, 58, 59]. Carbapenems were the best antibiotics for treating infections caused by MDR strains of *A. baumannii*. However, the frequency of isolates resistant to carbapenems is increasing worldwide[60, 61, 62].

In our study, air samples were uncultivable; may due to the presence of *A.baumannii* in air but in very low concentrations. To confirm the presence of A.baumannii in air as a possible reservoir of this bacteria, the plates should opened for prolonged time or other techniques depends on collect air samples in broth media to concentrate the bacteria may use.

Most of the *A. baumannii* isolates were found in the age group ≤15 years followed by ≥60 years, similar to previous studies [63,64].It has been noticed that a patient's age significantly influences their susceptibility to contracting a *A. baumannii*infection, in other words age considered one of the risk factors for *A. baumannii* infections. In addition, both age groups could described as immunocompromised patients,thus they are at risk to colonized or infected with *A. baumannii*.

Isolates obtained fromin-patients were higher than those collected from out-patients. The highest percentage of clinical and environmentalisolates of *A. baumannii*were found in ICU ward (45.8% and 75.9%, respectively) suggesting that ICU could be a potential source of *A. baumannii*, similar to previous reports[36,65].
In our study, the highest percentage of clinical *A. baumannii*isolates was observed in wounds (41.7%),followed by blood (25%). We observed that the majority of hospital isolates were obtained from patient’s beds followed by sinks, and that tables and door handles could be a source of A. baumanniiinfections. It is been shown that that hospital

environments are considered as a possible source for *A. baumannii* transmission, highlighting the importance of implementing control protocols to limit the transmission of *A. baumannii* through hospital environments [66,23].

In ourstudy, all isolates showed high resistance rates to commonlyused antibiotics, except for COT which has the lowest resistance rate, therefore,COT seems to bethe most effective antibiotic against clinical and environmental*A. baumannii*isolates with susceptibility rates of 84.6% and 58.6%, respectively, this is in agreement with Abdar et al., who reportedhighest susceptibility rate of COT [67].

We found that resistance rates were higher in environmentalthan clinical isolates, this highlights the importance of implanting infection control measures to decrease the contamination with these resistant isolates.However, both groups were 100% resistant to CTR, in addition, environmental isolates were 100% resistant to AK, GEN, IMP, This is in agreement with Janbakhsh et al [68].Antimicrobial susceptibility testing of the clinical isolates showed high resistance rate to AK and GENand both groups showed high resistance rate to CIP and CAZ which is similar to what reported in a previous study [36].

Raroet al. reported high resistant rates of environmental and clinical *A. baumannii* isolates to Carbapenems (MEM and IMP) similar to our findings [36], these results reflect considerable concern and a limitation in the options of treatment for*A. baumannii* infections ascarbapenems were the last choice for treatment of nosocomial infections results from *A. baumannii*The global emergence of CRAB strains could be as a result of misuse and overuse of these antibiotics for hospitalized patients [69].

The increasing in life-threatening CRAB *A. baumannii*infections leads to the use of polymyxins that should be used with calculated doses because of its nephrotoxicity and neurotoxicity adverse effects[70],particularly theCL which is considered the last choicein the treatment of CRAB and considered as a rescue therapy for severe infections caused by CRAB.

CL overuse of CL and Carbapenems will increase the resistance rates, worsening the situation by making it more difficult to eradicate MDRAB infections and increasing mortality rate [69]. Other studies found that all of their isolates were sensitive to CL [24, 68, 29]. Kaur et al. study reported that 97.4% of clinical *A. baumannii* isolates were sensitive to CL [71]. However, in our study, only 6.9% of *A. baumannii* isolates were sensitive to CL while the majority of isolates displayed intermediate sensitivity pattern, these results of CL sensitivity test performed by disk diffusion method might be unreliable due to the poor diffusion of CL on agar as a result of large CL molecule [72].CLSI reported that broth microdilution is the only approved MIC method for CL. clinical data of CLSI found that it has limited clinical efficacy, even with intermediate sensitivity and it should be used in combination with other active antibiotics [55]. COT is one of the few antibiotics that still effective against sever CRAB infections especially in combination with CL, Nepka et al. found that COT and CL combination were most effective against CRAB than using each antibiotic separately[73].

Shali et al. found that 37% of *A. baumannii* isolates were MDR [38], While Raro et al. reported 98.8%environmental and 97.8% of clinical *A. baumannii* isolates were MDR [36].However,our analysis,showed that 73.9% of clinical and 100% of environmental*A. baumannii* isolateswere resistance to at least one antibiotic in three or more antimicrobial classes.

This increase in the MDR strains might be due to the acquisition of mobile genetic materials such as integrons, plasmids and transposons that is considered is worldwide problem and can accomplish antibiotic resistance by altering gene expression [60].

plasmids are a major contributor to dissemination of antibiotic-resistance and can be transmitted from parent cell to progeny vertically or by conjugation horizontally. It has genes results in antibiotic resistance directly or within other elements as IS sequences or transposons. In addition, plasmids code the transfer genes needed for mobilization from the donor to a recipient cell. Therefore, antibiotic resistance as a results of plasmid transfer is a growing concern as it results in a limited availability of treatments for MDR and XDR A.baumannii[74].

Lastly, we performed genetic relatedness analysis by RAPD-PCR which revealed 4 different clusters among the isolates.The similar molecular pattern of environmental and clinical isolatescollected during different time periods from different wards (as shown in the cluster D),suggesting that the isolates had acommon source and disseminated among different hospital wards and clinics by patients transfer or mediated by medical staff or medical equipment resulting in cross contaminationwithin the hospital [7].

We observed Strains with the same RAPD cluster, but having different antibiograms, similar to the findings of previous study [75],this was most probably due to acquisition of genetic materials of resistance genes.

RAPD-PCR is rapid, simple and has good discrimination power. Therefore it has been used widely for molecular typing of *Acinetobacterspp*. However, it has low reproducibility due to the variation susceptibility by DNA template quality and concentration, primer concentration, gel electrophoresis[76]. In our study we overcomevariation bystandardized these variablesand performing RAPD-PCR for all isolates in one PCR run.

4.2 Conclusions

Our study revealed high resistance rates among isolates and high prevalence of MDRAB and CRAB in Alia hospital. The high prevalence of MDRAB and CRAB within the hospitals particularly in ICU as possible reservoir for resistant *A. baumannii*, thus, a source of nosocomial infections, is considered a public health concern. The detection of *A. baumannii* in different hospital environments, showed that hospital environments are important for MDRAB and CRAB strains dissemination and resulting in nosocomial infections.Thus, emphasized that supporting control measures as good cleaning and disinfection of hospital environment, hand hygiene, active surveillance and patient isolation may prevent outbreaks of MDRAB in hospital wards particularly ICU.

4.3 Recommendations

Continuous monitoring for the detection of MDR *A. baumannii* is essential to control its dissemination, reduce outbreaks and optimize the therapy. In addition, continuous monitoring of antibiotic resistance pattern and rigorous commitment to infection control policies are the way to prevent continuous dissemination of MDRAB and CRAB.

4.4Ethical considerations

Ethical approval was taken from Research Ethics Sub-committee of Faculty of Health Professions - Al-Quds University.

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