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MOLECULAR STUDY OF CARBAPENEMS RESISTANT *ACINETOBACTER BAUMANNII* ISOLATED FROM CLINICAL SOURCES

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MOHAMMED ADNAN FADHIL AL-NAJJAR

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MOLECULAR STUDY OF CARBAPENEMS RESISTANT *ACINETOBACTER BAUMANNII* ISOLATED FROM CLINICAL SOURCES

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December 2022

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

MOLECULAR STUDY OF CARBAPENEMS RESISTANT *ACINETOBACTER BAUMANNII* ISOLATED FROM CLINICAL SOURCES

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December 2022

Acinetobacter baumannii is one of the most important causes of nosocomial infections. The aim of this thesis is to identify *A. baumannii* from different clinical sources in Iraq hospitals by culture and PCR methods, to determine the antibiotic resistance/sensitivity status and minimum inhibitor concentration against isolates by disc diffusion to investigate the presence of carbapenem resistance genes by PCR method. We detected 64 (53.3%) *A. baumannii* from 120 swaps (burn, wound, blood, urine, sputum) samples by morphological, biochemical and PCR tests. The antibiotic susceptibility test showed that *A. baumannii* isolates had the resistance to Cefotaxime at 100% and the lowest resistance to Ampicillin/ Sulbactam at 40.6%. It was determined that the MIC value of levofloxacin was 8 µl and 2 µl, and amikacin was 512 µl and 16µl for isolates 1 and 2, respectively. Carbapenem-resistant was determined 25 (%50) *A. baumannii* isolates in the MHT test, 20 (%40) *A. baumannii* isolates in the Carba NP test. We found the OXA-58 gene in 2 (%4.0), isolates, the blaOXA-51 gene in all (%100), isolates OXA-40 gene in 12 (%24) isolates, the OXA-24 gene in 14 (%28) isolates, OXA-23 gene in 36 (%78.1) isolates, ISA-BAL gene in 46 (%90) isolates and no find OXA-143 gene from 50 *A. baumannii* isolate by PCR.

2022, 60 pages

Keywords: Antibiogram, β-Lactam, *bla*OXA, Carbapenemase genes

ÖZET

KLİNİK KAYNAKLARDAN İZOLE EDİLEN KARBAPENEMLERE DAYANIKLI *ACINETOBACTER BAUMANNII*'NİN MOLEKÜLER ÇALIŞMASI

Mohammed Adnan Fadhil AL-NAJJAR
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Aralık 2022

Acinetobacter baumannii nozokomiyal enfeksiyonların en önemli nedenlerinden biridir. Bu tezde, Irak'taki hastanelerden elde edilen farklı klinik örneklerde *A.baumannii* bakterisinin kültür ve PZR ile tanımlamak, izolatların antibiyotiklere direnç/duyarlılık durumunu ve MIK değerlerini disk difüzyon belirlemek ayrıca karbapenem direnç genlerinin varlığını PZR ile araştırmak amaçlanmıştır. Kültür, biyokimyasal ve PZR test sonuçlarına göre 120 örneğin (yanık, yara, kan, idrar, balgam) 64'ünde (%53,3) *A. baumannii* identifiye edilmiştir. Antibiyotik duyarlılık testi, 64 örneğin (%53,3) sefotaksim'e %100 oranında en yüksek dirence sahip olduğu ve Ampicillin/ Sulbaktam'a %40,6 oranında en düşük dirence sahip olduğu belirlenmiştir. sonuçlarına göre amikasine karşı izolat 1 ve 2'nin MİK değeri sırasıyla 8 µl/ml ve 2 µl/ml, levofloksasine karşı sırasıyla 512 µl/ml and 16 µl/ml olarak belirlenmiştir. Karbapenem direnci MHT testinde 25 (%50) *A. baumannii* izolatında, Carba NP testinde 20 (%40) *A. baumannii* izolatında saptandı. Yapılan PCR testi ile 50 *A. baumannii* izolatında OXA-58 geni 2 izolatta (%4,0), blaOXA-51 geni tüm izolatlarda (%100), OXA-40 geni 12 (%24) izolatta, OXA-24 geni 14 (%28) izolatta, OXA-23 geni 36 (%78,1) izolatta, ISA-BAL geni 46 (%90) izolatta belirlenirken, OXA-143 geni belirlenmemiştir.

2022, 60 sayfa

ANAHTAR KELİMELER: Antibiyogram, β-laktam, *bla*OXA, Karbapenemaz genleri

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Mohammed Adnan Fadhil AL-NAJJAR

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LIST OF SYMBOLS

%	Percentage
°C	Celsius
bp	Base pair
F	Forward sequences
kb	Kilo base
ng	Nanogram
nm	Nanometer
R	Reverse sequences
μL	Microliter

LIST OF ABBREVIATIONS

$BaCl_2$	Barium chloride dehydrate
CLSI	Clinical laboratories standards institute
CRAB	Carbapenem resistant *Acinetobacter baumannii*
DW	Distilled water
ESBL	Extended-spectrum β-lactamases
H_2S	Hydrogen sulfide
ICU	Intensive care unite
IMP	Imipenem hydrolyzing βlactamases
KOH	Potassium hydroxide
LBA	Luria-bertani agar
MDR	Multiple drugs resistance
MHA	Muller-hinton II agar
MHT	Modified hodge test
MIC	Minimum inhibitory concentration
OMP	Outer membrane protein
OXA	Oxacilin
PBPs	Proteins that bind pencillin
PCR	Polymerase chain reaction
TBE	Tris-borate-edta
TSI	Triple sugar iron
TSIA	Triple sugar iron agar
WHO	World health organization
XDR	Extensively drug-resistant

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1. INTRODUCTION

The *Acinetobacter baumannii* is a Gram-negative, opportunistic bacterium has become a predominant cause of healthcare-associated infections worldwide, is responsible for 2–10% of all infections acquired in hospitals (Antunes *et al.* 2014). As a result of its unique characteristics and exceptional potential to rapidly acquire resistance determinants to a broad spectrum of antimicrobials, this microorganism has emerged as a major concern for clinicians around the world, causing infections as a significant factor in ventilator-associated pneumonia and bacteremia, and less frequently meningitis, skin and soft tissue infections, urinary tract infections, septicaemia,endocarditis, burns, and wound infections in hospitals (Dahdouh *et al.* 2017). Because of the grave dangers it poses to public health, *A. baum*annii was designated as a "priority pathogen" (a group of bacteria that are resistant to many antibiotics) accoding to World Health Organization (WHO) (Anane *et al.* 2020). Immunosuppression, burns, trauma, mechanical breathing, catheters, invasive medical procedures, prior antibiotic therapy, a prolonged hospital stay (>90 days), and underlying disorders like diabetes all increase the likelihood of contracting an *A. baumannii* infection (El-Shazly *et al. 2015*).

Patients with impaired immune systems are more likely to develop infections; this includes the old people, those in the (ICU), and those undergoing major invasive surgeriesl, the catastrophic effects of this infection, including mortality rates as high as 50%, the pathogen's capacity to live for extended periods on surfaces around patients, and the rise of multidrug-resistant strains all contribute to the pathogen's importance from an epidemiological perspective (Howard *et al.* 2012).

Multidrug-resistant *A. baumannii* is a prominent cause of nosocomial infections worldwide due to its ability to persist and survive in hospital settings and dry environments (Ghasemi *et al.* 2018). *A.baumannii* are resistant the families drug such β-lactams (Pencillins, Cephalosporins, Carbapenems, Monobactams), and Aminoglycosides, fluoroquinolones (Kyriakidis *et al.* 2021).

Bactericidal -lactam antimicrobials, known as carbapenems, have been demonstrated to be successful in treating life-threatening infections caused by bacteria with resistance to standard antimicrobials β-lactamase, some examples include biapenem, doripenem, imipenem, meropenem, ertapenem, and panipenem (Patel and Bonomo 2013). Serious nosocomial infections caused by *A. baumannii* are best treated with carbapenems (Sohrabi *et al.* 2012). There is currently widespread emergence of *A. baumannii* strains are resistant to the antibacterial drug carbapenem growing antibiotic resistance, as reported by hospitalized patients, is correlated with increased mortality rates during the previous five years, the most common mechanism of carbapenem resistance among *A. baumannii* isolates is the presence of class D -lactamases (Kuo *et al.* 2012).

Carbapenem resistance outer membrane proteins (CarO) changes or loss, are among the many carbapenem resistance pathways discovered for *A.baumannii* (Lee *et al.* 2010). While efflux alterations are a factor in *A. baumannii* resistance to carbapenems, they are not enough on their own to cause a noticeable resistance in the clinic (Coyne *et al.* 2011). The creation of carbapenem-hydrolyzing enzymes is largely responsible for the rapid rise in carbapenem-resistant bacteria that has occurred as a result of the antibiotic's widespread use in recent years, ambler Class D OXA-type carbapenemases include blaOXA-23-like (OXA-23, OXA-27, and OXA-49), blaOXA-40-like (OXA-24/40, OXA-25, OXA-26, and OXA-72), blaOXA-58-like (OXA-58, and OXA-96), blaOXA-143-like ISAba1 is the most often discovered insertion sequence (IS) upstream of OXA-type class D carbapenemases in *Acinetobacter*, and other IS may affect the expression of these genes (Nguyen and Pham 2020).

Aim of this study to detect the distribution of imipenem-resistant *A. baumannii* from different clinical sources so this study was aimed to evalute the prevelence of molecular mechanisms of resistance among carbapenem-resistant *A. baumannii* in Iraq hospitals identifying the various enzymes responsible for carbapenem resistance requires phenotypical and molecular characterization of carbapenem-resistant *A.baumannii* isolates.

2. LITERATURE REVIEW

2.1 Classification and History of the *Acinetobacter*

In 1911, beijerinck identified the bacterium now known as Acinetobacter as Micrococcus calco-aceticus, *Acinetobacter* is derived from the Greek word "akinetos," which means unable to move, because these bacteria do not have flagella to propel them *Acinetobacter baumannii*, *A. calcoaceticus, A. haemolyticus* and *A. lwoffii* are the most important species in clinical practice, Gram-negative bacteria belonging to the genus Acinetobacter have a DNA G+C concentration of 39% to 47%, bacilli size is range in from 0.9 to 1.6 m in diameter and 1.5 to 2.5 m in length, at $37°C$, Acinetobacter grows as colonies that are smooth, grayish white, and mucoid on solid media used in medical diagnosis, such as sheep blood agar and tryptic soy agar (Peleg *et al.* 2008).

This diverse group of bacteria has seen a fascinating, complex, taxonomic history since its initial description at the turn of the twentieth century, Acintobacters have been widely recognized and emerging as nosocomial pathogens since the 1980s, and as a result, the taxonomy of these bacteria has been revised and regularly updated since then (Dijkshoorn *et al.* 2007).

In medicine, *A. baumannii* is the most important clinical species, according to epidemiological data, this species is responsible for over 80% of all *Acinetobacter* infections, Antimicrobial resistance, colonization potential, and contact transmission are the primary threats posed by these microorganisms (Jaka-Loxha *et al.* 2013).

Some of the Acinetobacters that have been formally named are *A. baumannii, Acinetobacter calcoaceticus*, *Acinetobacte rhaemolyticus*, *Acinetobacter johnsonii, Acinetobacter junii,* and *Acinetobacter lwoffii*, this categorization was based on DNA-DNA hybridization tests (Vijayakumar *et al*. 2019).

A. baumannii Belongs to the Gamma proteobacteria class, Pseudomonadales order, and Moraxellaceae family genus *Acinetobacter* (Van der Kolk *et al.* 2019).

The *A. baumannii* is aglucose-non-fermentative, Gram-negative coccobacillus non motile, strictly aerobic, oxidase negative,catalase positive It is now recognized as a leading cause of nosocomial infections, which can lead to serious complications for hospitalized patients (wang *et al.* 2019). *A. baumannii* is a gram-negative coccobacillus that is nonmotile, strictly aerobic, oxidase negative, and catalase positive, it has recently emerged as a major source of nosocomial infections, which are associated with increased morbidity and mortality (Giannouli *et al.* 2013).

The morphology becomes more spherical in the stationary phase and they are 1.5–2.5 m in length and 1–1.5m in diameter and are frequently found in pairs or long chains, the suggested incubation for bacterial isolates is optimally between 37$°C$ to 42°C, where experiments have also shown *A.baumannii* recovery up to 44 $°C$ under aerobic conditions (Benoit *et al.* 2020). Most *Acinetobacter* species grew best at a temperature of 37$°C$, whereas the strain *A.baumannii* can survive at a temperature of 44 °C (Asif *et al.* 2018). Therefore, in media such macConkey agar and blood agar *A.baumannii* grow easily On MacConkey agar, it appear light or not lactose fermenting, appears as an opaque, elevated, smooth, and creamy colony in blood agar (Cools *et al.* 2019).

2.2 Pathogenesis of *A. baumannii*

Patients in critical condition are especially vulnerable to infection with *A. baumannii*, which is an opportunistic human pathogen, *A.baumannii* was once assumed to be harmless; nevertheless, its rapid development of multidrug, multiple drug, and even pandrug resistance phenotypes has made it a global concern in healthcare facilities (Giammanco *et al.* 2017).

This opportunistic pathogen mostly causes ventilator-associated pneumonia, community and nosocomial infections, as well as bloodstream, urinary tract, skin, and soft tissue infections, particularly in critically sick patients in intensive care units (ICUs) (Morris *et al.* 2019). Serious illness from *A. baumannii* occurs only in those with impaired immune systems, a lack of an effective treatment for antibiotic-resistant bacteria, however, can worsen symptoms and increase death (Tabah *et al.* 2012). The centers for disease control and prevention (CDC) consider *A. baumannii* to be a "severe" danger due to the widespread presence of MDR and XDR strains (Solomon *et al.* 2014). As a high-risk disease to human health, according to the world health organization, *A.baumannii* is a bacteria for which new antibiotics are urgently needed (WHO 2017).

Linens, curtains, bed rails, tables, sinks, doors, feeding tubes, and other medical equipment can all serve as fomites for *A. baumannii* if they come into contact with an infected person or colonizer, the majority of infections are spread through the use of contaminated respiratory support equipment, suction devices, and intravascular access devices (Jung *et al.* 2015). Unless it is isolated from people with comorbidities such premature infants with low birth weights or the elderly with persistent illnesses like cancer, *A. baumannii* is regarded to be a low-virulence pathogen, major risk factors for developing an *A. baumannii* infection include: intensive care unit (ICU) admission, prolonged hospital stay, mechanical breathing, intravascular device use, advanced age, immunosuppression, past broad-spectrum antibiotic therapy, prior sepsis, and enteral feedings (Islahi *et al.* 2015).

2.3 Virulence Factors of *A. baumannii*

Many virulance factors are present in *A.baumannii*, which include lipopolysaccharides, OmpA, efflux pumps, hemolytic factors, iron acquisition systems, and hemolytic factors, can stimulate the host immune system or cause bacterial adhesion to epithelial cells (Ali *et al.* 2017). Outer membrane protein A is the most abundant *A. baumannii* outer membrane protein (OMP) and one of the most comprehensive analyses of virulence factors, which are crucial to bacterial adaptability and disease in host cells (Lee *et al.* 2010). Inducing an inflammatory response in human monocytes in response to *A. baumannii* is believed to be a major contributor to the pathophysiology of infection, and lipopolysaccharide isolated from *A. baumannii* has been demonstrated to be a powerful inducer of pro-inflammatory cytokine production (Gordon and Wareham 2010)

By decreasing the antibiotic concentration at the site of infection, efflux pumps contribute to the latter mechanism and make bacteria less susceptible to the chemical, all live cells have efflux transporters, which remove harmful organic compounds from the cell (Blanchard *et al.* 2014). The virulence factors of *A.baumannii* are given in Figure 2.1.

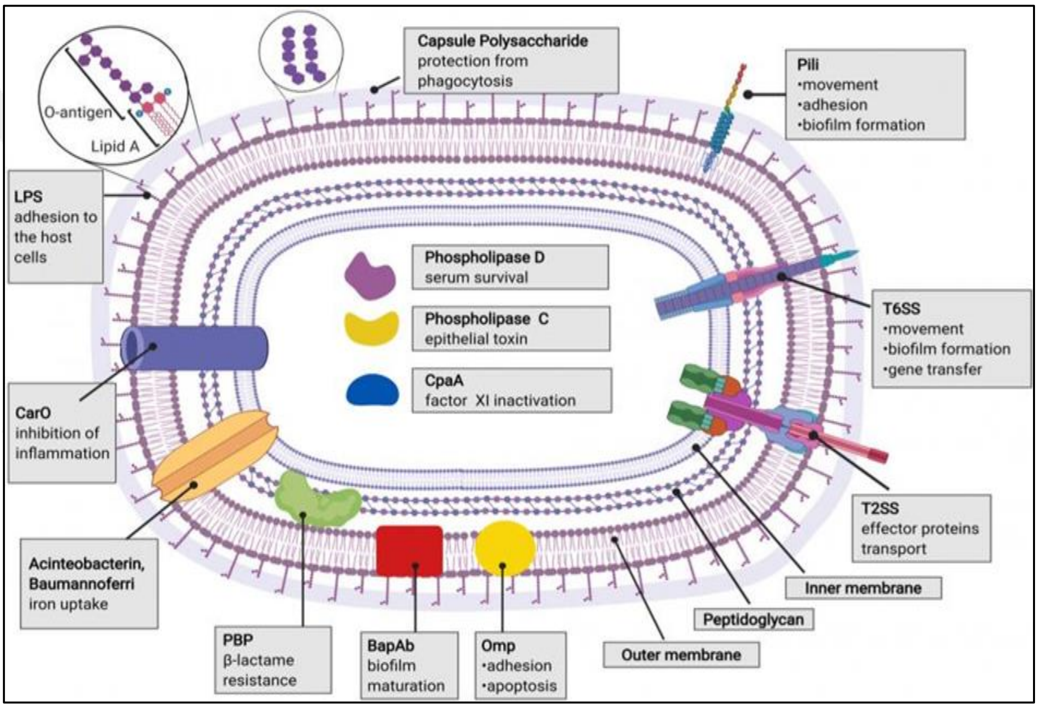

Figure 2.1 The virulence factors of *A.baumannii* (Blanchard *et al.* 2014)

2.4 Resistance β–Lactam Antibiotics

Over 50% of all of the clinically-available antibiotics are β-lactam containing compounds due to low manufacturing costs, minimum toxicity, and high efficacy (Wilke *et al.* 2005). Such as penicillins, cephalosporins, carbapenems and monobactams (Walsh 2000). This β-lactam Antibiotics chemical structure showed in Figure 2.2.

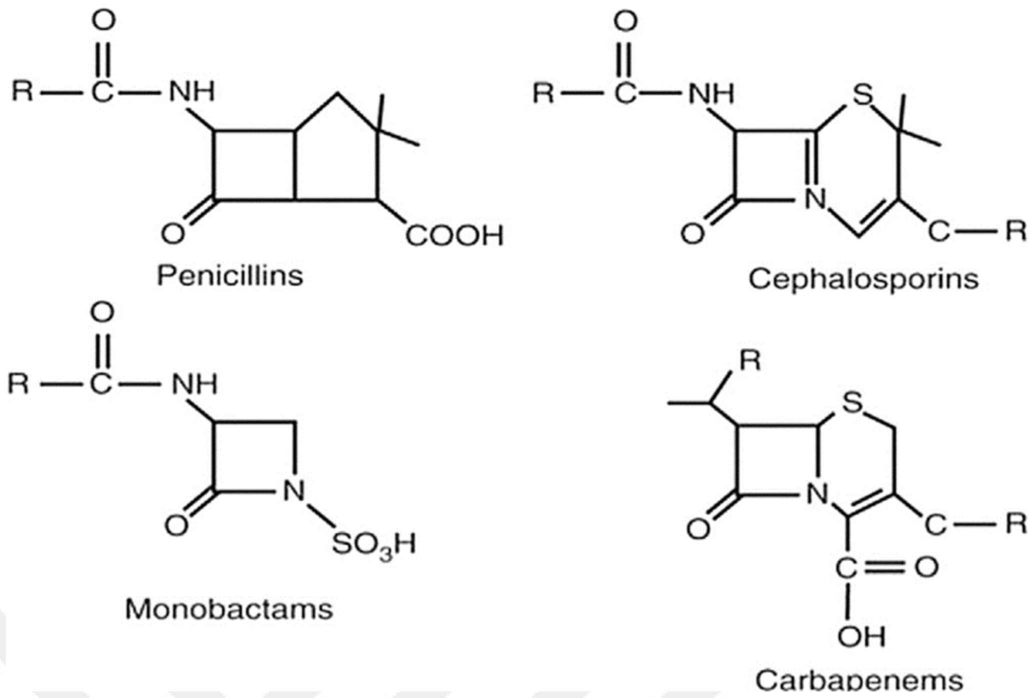

Figure 2.2 Structure of β-lactam antibiotics (Walsh 2000)

Inhibiting enzymes involved in cell wall formation is how beta-lactam medicines kill bacteria, thus, the lysis of cells occurs due to the lactam-mediated suppression of transpeptidation (Drawz and Bonomo 2010). The carbapenems are a subgroup of β-lactam antibiotics that are efficient against vast variety of bacteria that are Gram-positive and negative, both aerobes and anaerobes, among β-lactam antibiotics, they show the broadest effect range, carbapenems distinguish from penicillins throughout the presence of carbon at position C1 rather than sulphur and in the holding of a covalent bond with C2 and C3 in the five member ring, (Nowak and Paluchowska 2016). Mechanisms of their action are involved in the structure and biosynthesis of the bacterial cell wall as other βeta-lactam antimicrobials, carbapenems enter *A.baumannii* via outer membrane proteins often labeled porins traverse the periplasmic gap and bind to penicillin binding protein, transpeptidases and glycosyltransferase enzymes answerable for the creation of peptidoglycan in the bacterial cell wall are defined as pencillin binding proteins (PBPs), when carbapenem associated with this enzyme allows the peptidoglycan to be weaken and can result in a cell bursting due to osmotic pressure (Nowak and Paluchowska 2016).

The first carbapenem allowed for usage in 1985 was imipenem, a safe form of thienamycin detected in 1970 to be made by using Streptomyces cattleya, recent advances in 1993 meropenem was utilized, ertapenem was in 2001 and lastly doripenem at 2007 (Livermore 2012).

Imipenem should be used in combination with cilastatin because a dehydropeptidase enzyme hydrolyzes imipenem into a nephrotoxic metabolite in the human kidney, but cilastatin is an inhibitor of dehydropeptidase that inhibits the production of such a nephrotoxin metabolite meropenem, however, is stable against human dehydropeptidase and does not require coformulation of cilastatin (Wanger *et al.* 2017). Meropenem is an intravenously administered antibacterial medication, meropenem has been shown to have marginally higher success rates, and however, meropenem has no effect on Carbapenem resistant *A. baumannii* CRA, just like imipenem (Shah and Isaacs 2003).

Imipenem with meropenem's effectiveness against a wide range of illnesses, like infections inside the abdominal, infections of cutaneous structure, the community acquired pneumonia, hospitalized pneumonia, aggravated inflammation of the urinary tract, meningitis (only used meropenem) has been studied in comparative clinical trials, therefore, carbapenems are regarded among the most appropriate drugs for the treatment of pathogenic bacteria and a major public health problem is the increase and spread of resistance to these antibiotics (Codjoe and Donkor 2017).

Death rate linked with intrusive *A. baumannii* infection is large, particularly in cases that are carbapenem-resistant,Carbapenem-resistant crude mortality with *A. baumannii* range from 16% to 76%, compared to 5% to 53% with infections carbapenem-susceptible (Lemos *et al.* 2014). Excessive carbapenem resistance is due to over-expression of efflux pumps, genetic variations of penicillin binding proteins (PBP), synthesis of some enzyme for example carbapenemase and loss of porin expression (Rosales-Reyes *et al.* 2017).

The Ambler classification of β-lactamases is the most common system for organizing this family of enzymes, and it separates lactamases into four groups (A, B, C, and D) based on their amino acid sequences (Hall *et al.* 2005). Clinically important β-lactamases produced by select gram-negative organisms showed in Table 2.1

Table 2.1 Clinically important β-lactamases produced by gram-negative organisms

Ambler Classification	Representative Enzymes	Some Notable Organisms
Class A	ESBLs[1]	*Escherichia coli, Klebsiella* spp, *Proteus mirabilis*
	Carbapenemases (KPC[a])[2]	*Klebsiella pneumoniae, E. coli, Klebsiella oxytoca, Serratia marcescens, Enterobacter* spp, *Citrobacter freundii*
Class B	Carbapenemases, metallo-β-lactamases	*K. pneumoniae, E. coli, K. Oxytoca S. marcescens, Enterobacter* spp, *C. freundii*
Class C	Cephalosporinases (AmpC)[3]	Inducible chromosomal AmpCs: *Enterobacter* spp, *C. freundii, S. marcescens, Morganella morganii, Providencia stuartii*
	-	Plasmid-mediated AmpCs: *K. pneumoniae, E. coli, Salmonella enteritidis*
Class D	Carbapenemases (OXA)[4]	*A. baumannii, Pseudomonas aeruginosa, E. coli, K. pneumoniae, P. mirabilis, C. freundii*

1 Extended-spectrum β-lactamases, 2 Klebsiella pneumoniae Carbapenemases, 3 Ambler molecular classification cephalosporineses, 4 Oxaxilin

Carbapenemases, capable of hydrolyzing carbapenems and other betalactams (Queenan and Bush 2007). Before they have a chance to have an effect, these enzymes degrade antibiotics, carbapenemase encoding genes can be found both on chromosomes and on mobile genetic components like plasmids, this mobility element enables the resistance inducing gene to be easily transferred (Perez and Van Duin. 2013).

Carbapenemases classified into non metallo carbapenemases (class D zinc independent) and metallo carbapenemases based on their reliance on divalent cations for enzyme activation (class B zinc dependent), the class D ambler is known as oxacillinases because oxacillin is hydrolyzed faster than the benzylpenicillin rather than the other class that low hydrolysis of oxacillin (Jeon *et al.* 2015).

In particular, OXA-23, OXA-24, OXA-58, OXA-143, and intrinsic OXA-51 have been reported in several groups of oxacillinases in carbapenem hydrolysis found in *A.baumannii*, around 1985, shortly after the introduction of carbapenems acting as therapeutic agents, the OXA23 gene was identified within *A.baumannii* in Scotland country (Zhao et al. 2019). It was suggested that it originated from the *Acinetobacter* radioresisten chromosome, where it was mobilized via *A.baumannii* ISAba1, in a massively resistant *A.baumannii* plasmid the OXA58-like gene was located in France (2003) and additional six variants were discovered to date, in Africa, United States, Europe, Australia and South America, the OXA-58 gene was already linked with outbreaks in hospital (Zhao *et al.* 2019).

In the Spain recovered CRAB chromosome, the *OXA*24 gene was find out, subsequently in hospitals around the world, discovered CRAB strains carrying that gene and its variants mostly on small plasmids, this oxacillinase group includes of eight close relatives, with OXA25, OXA26, OXA40/24 and OXA-72 those are the most popular variants, and from other families the OXA143 and OXA-235 have also been associated in some countries with carbapenem resistant *A. baumannii* (CRAB) occurrences, but they are less frequently known and are generally considered to be mild causes of resistance to *A.baumannii* carbapenem (Hamidian and Nigro 2019).

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Intrinsic bla OXA-51 are the largest of the β lactamases of the OXA-type, in *A.baumannii* isolates, the OXA-51 enzyme was first identified, in 1996, it was obtained in Argentina (Brown *et al.* 2005).

This *OXA* 51-group baseline development does not result in clinically relevant resistance to carbapenem, the acquiring of a stronger upstream advocate of the OXA 51 by ISAba1 transposition can result in clinically significant levels of resistance to carbapenem (Viehman *et al.* 2014). Class B is a metallo-carbapenemase that splits the ring of β-lactam,There are two primary metallo lactamases found in *A. baumannii*, and they are both encoded by the verona integrin, the verona integron-encoded metallo lactamases (VIM) and the imipenem hydrolyzing lactamases (IMP) (Queenan and Bush 2007).

2.5 Carbapenemases (OXA) and Some Spesificaitons

In 1985, an *A. baumannii* strain was obtained in Scotland, and it was in this strain that the enzyme OXA-23 (then known as ARI-1) was initially discovered (Lee *et al.* 2010). The genes for OXA-23-like enzymes have been found on both chromosomes and plasmids in various *Acinetobacter* species and *Proteus* mirabilis isolates, it was proven that the enzyme was able to impart resistance to imipeneme showing in the Table 2.2 (Bogaerts *et al.* 2006).

The *bla*OXA-23-like genes have a G+C content of 37.6-37.9% (Bergogne and Towner 1996). This percentage lies beyond the normal range of *A. baumannii's* genome, which is between 39 and 47%, suggesting that these genes originated in a different species, evidence that the OXA-23-like enzymes may have evolved in this species comes from the identification of the *bla*OXA-23, *bla*OXA-102, *bla*OXA-103, *bla*OXA-105, *bla*OXA-133, and *bla*OXA-134 genes on the chromosomes of several *Acinetobacter* radioresistens isolates (Poirel *et.al* 2008). Isolates encoding OXA-23 have been discovered all over the globe, and they are commonly connected to outbreaks of illness in hospitals showing in the Table 2.2 (Villegas *et al.* 2007).

Table 2.2 The class D *OXA* type β-lactamases found in *A. baumannii*

Group	Group Members	Location
***OXA*-23 like**	OXA-23, OXA-27, OXA-49, OXA102, OXA-103, OXA-105, OXA133, OXA-134	Chromosomal , plasmid (Afzal-Shah *et al.* 2001)
***OXA*-24 like**	OXA-24, OXA-25, OXA-26, OXA-72	Chromosomal and plasmid (Turton *et al.* 2006)
***OXA*-51- like**	OXA-51, OXA-64 to OXA-71, OXA-75 to OXA-80,OXA-82 to OXA-84,OXA-86 to OXA-95, OXA-98 to OXA-100, OXA-104, OXA-106 to OXA-113,OXA-115 to OXA-117, OXA-120 to OXA-127	chromosomal (Koh *et al.* 2007)
***OXA*-58 like**	OXA-58, OXA-96, OXA-97	Chromosomal , plasmid (Mendes *et al.* 2009)

The OXA-*24*-like enzymes*:* Isolates taken from Belgium and Spain, respectively, in the years 1995 and 1997 led to the discovery of two more enzymes that bear the names OXA-25 and OXA-26, both of these enzymes share more than 99 percent of *OXA*-24's amino acid sequence (Turton *et al*. 2006). An enzyme with the designatio*n* OXA-72 and the sequence of which was published online in 2004 was found in an isolate from Thailand, this enzyme had an identity with OXA-24 that was more than 99 percent across all of its amino acids, these four enzymes make up the category of enzymes known as OXA-24-like enzymes (Afzal-Shah *et al.* 2001).

The OXA-*51*-like enzymes*;* OXA-51-like -lactamases are a family of enzymes that are both essential and widespread *in A. baumannii* (Turton *et al.* 2006, Bonnin *et al.* 2011). OXA-51, the group's pioneering enzyme, was discovered in 1996 in Argentinean isolates and initially described in 2004 (Brown *et al.* 2005). and ever since then, quite a few more enzymes that are connected to it have been found, when we first started working on this study, the OXA-51-like group was comprised of 32 different enzymes, there have been a total of sixty different OXA-51-like enzymes discovered so far, because of this, they are among the most genetically varied -lactamase families (Turton *et al.* 2006). The class D OXAtype β-lactamases found in *A. baumannii* showed in Table 2.2. ,genes have a G+C content between 38.4 and 39.9 percent, which is quite close to the 39 to 47 percent found in the *A. baumannii* genome this lends credence to the idea that these genes have always been a part of *A. baumannii* (Bergogne and Towner 1996).

The OXA-*58* like enzymes*:* In 2003, the enzyme OXA-58 was discovered in a French clinical isolate of *A. baumannii* (Poirel *et al.* 2005). The enzyme was found to be localized to a plasmid that was thirty thousand bytes in size, it has an amino acid identity of 48 percent with OXA-23 and 47 percent with OXA-24, respectively, since then, and two enzymes that are very closely related to OXA-58 have been discovered, an *A. baumannii* strain that was isolated in 1996 from a hospital in Singapore was found to contain a beta-lactamase known as OXA-96, methionine, rather than isoleucine, can be found at position 161 in this enzyme, setting it apart from the OXA-58 enzyme, additionally, an enzyme known as OXA-97 that differs from OXA-58 in that it contains a glycine instead of an alanine at position 53 the OXA-58-like enzyme group is made up of these three different enzymes (Koh *et al.* 2007).

A number of OXA-58 like enzymes have been identified in different *Acinetobacter* species, these enzymes are often carried by plasmids, although their locations on the chromosome have also been identified (Bogaerts *et al.* 2006). The G+C content of the *bla*OXA-58-like genes ranges from 37.4 to 37.5 percent, this is less than the G+C composition of the *A. baumannii* genome (which sits at between 39 and 47 percent) (Bergogne-Berezin and Towner 1996). Showing that these genes could have come from a different species in the first place, isolates carrying *bla*OXA-58-like genes have been reported most often from Europe, however, isolates carrying these genes have also been identified from South America, North America, Asia Minor, Asia, and Australia (Lee *et al.* 2010). The outbreaks with OXA-58-like genes were reported in France and Greece (Bonnin *et al.* 2011).

The OXA-143 like enzymes: OXA-143, a novel OTC subgroup, was found in a carbapenem-resistant *A. baumannii* strain obtained in Brazil (Higgins *et al.* 2009). This enzyme, which was found on a plasmid of around 30 kb, shares 88% of its amino acid sequence with OXA-40, 63% with OXA-23, and 52% with OXA-58 (Higgins *et al.* 2009). The only other member of this recently described cluster of CDHLs is OXA-143, which has so far only been found in *A. baumannii* strains isolated from hospitals in the Brazilian states of Sao Paulo and Rio de Janeiro (Gionco *et al.* 2012*)*.

3. MATERIALS AND METHODS

Isolation and identification studies of *A. baumannii* were carried out from a total of 120 samples (burns, wounds, blood, urine, and sputum) brought to a private laboratory belonging to health institutions in Baghdad. 16 different antibiotic discs were used to identify bacterial carbapenemase resistance; the presence of *bla*OXA-143, *bla*OXA-58, *bla*OXA-51, *bla*OXA-40, *bla*OXA-24, *bla*OXA-23 and ISA-BAL genes in 50-carbapenemase producing isolates was investigated by polymerase chain reaction (PCR).

3.1 Materials

3.1.1 Sample collection

Between November 2021 and February 2022, 120 clinical specimens were collected, including randomly from wound infections, burns, sputum, blood and urine samples. Samples from patients of both sexes aged 8 to 70 years, were completed at the end of February 2022. Laboratory samples are given in Table 3.1 according to their sources and gender.

Table 3.1 Sources and number of samples

Example Source	Number of samples	Male	Female
Wound infections	26	12	14
Burns swab	44	19	25
Sputum swab	20	12	8
Blood	20	6	14
Urine	10	4	6
Total	120	53	67

3.1.2 Instruments and equipment

Instrument and equipment used in the study with their suppliers given in Table 3.2.

Table 3.2 equipment and Instruments used in the study with their suppliers

NO	Equipment	Company	Origin
1.	Autoclave	Gallenkamp	England
2.	Air flow cabinet	Liofilchem	Italy
3.	Dry block thermostat built Bio TDB-100	Bio San	Germany
4.	Micropipette, Biopette (0.5-10 μL, 2-20 μL, 10-100 μL, 100-1000 μL)	Bio San	Germany
5.	Burner	Amal	Turkey
6.	Centrifuge	Hettich	Germany
7.	Combi-spin	Biosan	Lativa
8.	Deep Freezer	Sanyo	Japan
9.	Digital camera	Sony	Japan
10.	Electrophoresis Unit	CBS, Scientific	USA
11.	Gradient Thermocycler	Eppendorf	Germany
12.	Hot plate with magnetic stirrer	Gallenkamp	England
13.	Incubator	Gallenkamp	England
14.	Mini-centrifuge	Bio San	Germany
15.	Microwave Oven	LG	Korea
16.	Mixer	Griffin	Germany
17.	PCR Thermal Cycler	Labnet	USA
18.	Nanodrop	Nabi	Korea
19.	Oven	Heraens	Germany
20.	Shaking Incubator	GFL	Germany
21.	Transport swap	GFL	Germany
22.	UV. transmission	Vilber lourmat	Farance
23.	Vortex	Griffin	England
24.	Water bath	Sony	Gallenkamp
25.	96 well microplate (Cito-test)	Jiangsu	China
26.	Gel electrophoresis	Intron	Korea

3.1.3 The chemicals materials

The chemicals materials used in this study and their manufacturer. The suppliers are listed in Table 3.3.

Table 3.3 Chemicals used in the study with their suppliers

No.	Chemicals and other materials	Company	Origin
1.	Absolute ethyl alcohol (99.9%)	Diamound	France
2.	Agarose	Promega	USA
3.	Agar agar	Himedia	India
4.	Crystal violet	Fluka	England
5.	Safranine	Fluka	England
6.	Iodine	BDH	England
7.	DNA Loading dye	Promega	USA
8.	Ethidium bromide (10mg/mL)	Promega	USA
9.	Sodium chloride (NaCl)	BDH	England
10.	Sulfuric acid (H2SO4)	BDH	England
11.	Zinc sulfate (ZNSO4)	Promega	USA
12.	Sodium hydroxide (NAOH)	Promega	USA
13.	potassium hydroxide (KOH)	Pharmacia	Sweden
14.	Barium chloride dehydrate (BaCl2.2H2O)	BDH	England
15.	Glycerol	BDH	England
16.	Methyl red	GFS	Canada
17.	Peptone water	Mast	UK
18.	Phenol red	Oxoid	England
19.	Normal Saline (Sterile)	Pioneer	Iraq
20.	DNA Marker (100-1000) bp	NEB	England
21.	Free Nuclease Water	NEB	England
22.	Normal Saline	Bioner	Iraq
23.	RedSafe dye	Intron	South Korea
24.	TAE Buffer (50x)	Carl ROTH	Germany
25.	Isopropanol, 70% Ethanol	ROMIL pure chemistry	UK
26.	Chloroform	LiChrosolv	Germany

3.1.4 Culture media

All ready-made media that was used in this study were listed in Table 3.4. Processed according to instructions from the manufacturing company and autoclaved for 15 minute at 121 $°C$ at 15 pound per square inch.

Table 3.4 Culture media used in this study

No.	Media	Company	Origin
1.	Brain heart infusion agar	Liofilchem	Italy
2.	Brain heart infusion broth	Liofilchem	Italy
3.	Chrom agar col-APSE base	Rambach	France
4.	Chrom agar *Acinetobacter* base	Rambach	France
5.	Muller-Hinton II agar (MHA)	Liofilchem	Italy
6.	MacConkey agar W/O Crystal Violate	Liofilchem	Italy
7.	Luria-Bertani (LB) agar	Liofilchem	Italy
8.	Triple Sugar Iron Agar (TSIA)	Himedia	India
9.	Simmon citrate agar	Himedia	India
10.	Urea Agar base	Mast	UK
11.	Methyl red vogas proskauer (MRPV)	Himedia	India

3.1.5 Biochemical test solutions for bacterial diagnosis

Four reagent were used in this study for bacterial diagnosis. This reagents were explained under the following secition.

Catalase reagent (Promega, USA); The reagent was prepared from 3 % of hydrogen peroxide (H2O2). Oxidase reagent (Himedia,India): Preparation of the reagent involved dissolving 1 g of tetra-methyl-p-phenylenediamine dihydrochloride in 100 milliliters of deionized water, and the resulting solution was then stored in a dark bottle.

Methyl Red reagent (Himedia , India): to make the solution, 0.1 g of methyl red was dissolved in 300 mL of 95% ethanol, and the volume was brought up to 500 mL using water.

Vogas- Proskaur reagent (Mast, UK): Three milliliters of solution 1 and one milliliter of solution 2 were combined to make this reagent. To make Solution 1, 59 m of - naphthol was dissolved in 100 mL of 95% ethanol and the mixture was stored in a dark bottle until needed. Dissolving 40 g of KoH in 100 milliliters of D.W. yielded Solution

3.1.6 Antibiotics for antibiogram test

The antibiotics used in this study given Table 3.5.

Table 3.5 The antibiotics used in disc diffusion and btorh microdilution tests.

NO.	Antibiotic	Abbreviation	Doses	Company/ Origin
1.	Amikacin	AK	30 µg	Himedia
2.	Apicillin-Sulbactum	AMS	10/10 µg	Liofilchem
3.	Cefepime	FEP	30 µg	Liofilchem
4.	Cefotaxime	CTX	30 µg	MAST / UK
5.	Ceftriaxme	CRO	10 µg	Bioanalyse/Turkey
6.	Ceftazidime	CAZ	10 µg	MAST / UK
7.	Colistin	CL	10 µg	MAST / UK
8.	Ciprofloxacin	CIP	5 µg	MAST / UK
9.	Gentamicin	CN	10 µg	MAST / UK
10.	Imipenem	IPM	10 µg	MAST / UK
11.	Levofloxacin	LEV	5 µg	Himedia
12.	Meropenem	MEM	10 µg	MAST / UK
13.	Piperacillin	PRL	100 µg	Bioanalyse/Turkey
14.	Trimethoprim	TR	23.75 µg	Himedia
15.	Tetracycline	TE	30 µg	Bioanalyse/Turkey
16.	Tigecycline	TGG	15 µg	Bioanalyse/Turkey
17.	Amikacin antibiotic solution (For MIC)	AK	50 mg / mL	UK
18.	Levofloxacin antibiotic solution (For MIC)	LEV	50 mg / mL	Bioanalyse/Turkey
19.	Meropenem antibiotic solution	MEM	10 mg / mL	India

3.1.7 DNA extraction kit

Genomic BYF DNA Extraction Mini Kit (Cat no: 17045/ Intron biotechnology/Korea) used for DNA extraction in this study. The contents of this kit are list in Table 3.6.

Table 3.6 Content of genomic DNA extraction mini kit content

No.	Kit Content
1	MG Buffer
2	MW Buffer
3	Proteinase K4
4	ME Buffer

3.1.8 Primers for molecular detection of *A.baumannii* some genes

16 srRNA Primers designed by us and all primers synthesized by (Ligo) company. Primers used in *A. baumannii* molecular identification and detection of carbapenem resitance gene in this study given Table 3.7.

Table 3.7 Primers and molecular sized of PCR products for *A.baumannii* genes

Genes	Primers	Oligonucleotide sequence (5-3)	Product size/bp	Primer reference
16 *sr*RNA	Forward	5'-GCTAATAGATGAGCCTAAGTC- 3'	585 bp	Designed
	Reverse	5'-CAGGGTATCTAATCCTGTTTG- 3'		
Oxa-51	Forward	5'-TAATGCTTTGATCGGCCTTG - 3'	353 bp	(QADER, 2021)
	Reverse	5'-TGGATTGCACTTCATCTTGG- 3'		
Oxa-23	Forward	5'-GATCGGATTGGAGAACCAGA-3'	501 bp	(Chen, *etal*. 2019)
	Reverse	5'- ATTCTTGACCGCATTTCCAT- 3'		
Oxa-24	Forward	5'-ATGAAAAAATTTATACTTCCTATATTCAGC-3'	828 bp	(Askari *etal*. 2019)
	Reverse	5'-TTAAATGATTCCAAGATTTTCTAGC-3'		
Oxa-143	Forward	5'-TGGCACTTTCAGCAGTTCCT-3'	150 bp	(Chen, *etal*. 2019)
	Reverse	5'-TAATCTTGAGGGGGCCAACC--3'		
Oxa-40	Forward	5'-GGTTGTTGGCCCCCTTAAA-3'	246 bp	(Qi, *etal*. 2008)
	Reverse	5'-AGTTGAGCGAAAAGGGGATT-3'		
Oxa-58	Forward	5'-AAGTATTGGGGCTTGTGCTG-3'	599 bp	(Chen, *etal*. 2019)
	Reverse	5'-CCCCTCTGCGCTCTACATAC-3'		
ISA_BA1	Forward	5'-ATGCAGCGCTTCTTTGCAGG - 3'	390 bp	(Qi, *etal*. 2008)
	Reverse	5'- AATGATTGGTGACAATGAAG- 3'		

3.2 Methods

3.2.1 Samples collection

One hundred and twenty specimens were collected randomly from different clinical source included wound infections, burns, sputum, blood and urine samples from reference deferent patients of both gender, patients ranged in age from 8 to 70 years.

These specimens collected from different hospitals (Al-Kindi Hospital, Al- Kadhimiya General Hospital, Medical city Hospital, Al-Karama and Al-Yarmouk hospital) in Baghdad.

3.2.2 Prepare of culture media

All media (Brain heart infusion agar , Chrom agar col-APSE base , Chrom agar *Acinetobacter* base, Muller-Hinton II agar, MacConkey agar, Luria-Bertani (LB) agar, Triple Sugar Iron Agar , Simmon citrate agar, Urea Agar base) presented in Table 3.5 were made with the ingredients diluted in distilled water per the manufacturer's instructions (DW). A water bath boil was used to fully dissolve all ingredients once the pH was lowered to 7.2, media were autoclaved at 121 $°C$ for 15 min at 15 pounds per square inch and then placed in sterile Petri plates; alternatively, media were incubated at 37 $°C$ for 24 hours to guarantee sterility.

3.2.3 Isolation of *Acinetobacter*

All collected specimens were transported to the Laboratory site, then, it streaked under septic condition on MacConkey agar, and CHROMagar *Acinetobacter* medium incubated for 24 hours at 37ºC. On MacConkey agar plates isolated show red colonies on CHROMagar *Acinetobacter* medium isolated appear non-hemolytic opaque creamy colonies. The suspected colonies depending on the morphological bases were selected and further identification tests included the morphological and biochemical tests were carried out depending on procedure to diagnosis *Acinetobacter*.

A single colony of each isolate was examined by a light microscope using oil immersion after being fixed well and stained with Gram's stain to observe the bacterial cells, gram reaction, shape of cell and arrangement were observed (Fischbach 2006).

Morphological colonies was studied on MacConky agar, brain heart infusion agar and CHROM agar *Acinetobacter* medium, color, shape, size, and edge of colonies were recorded after over night incubation at 37$°C$ (Morgan *et al.* 2010).

3.2.4 Identification of *A. baumannii* by biochemical tests

Indole test; this assay determined whether or not the bacteria could manufacture the tryptophanase enzyme necessary to convert tryptophan into indole, pyruvic acid, and ammonia, after 24 hours of growth at 37 $°C$ in MacConkey agar broth, the bacteria were tested for their ability to produce tryptophanase by adding 10 drops of Kovac's reagent directly to the culture tube and gently shaking the contents, if a red ring formed at the top of the broth, the bacteria were successful (Sadauskas *et al.* 2017)

Oxidase test: The oxidase test detects the presence of the enzyme cytochrome oxidase, also known as indophenol oxidase, by a biochemical reaction if the reduced, colorless reagent is in the presence of an organism containing the cytochrome oxidase enzyme, it will undergo oxidation, producing a colorful by product (Shields and Cathcart 2010).

Catalase test: The catalase enzyme activity was measured using the slid technique, using a wooden stick, we transferred some pure growth to a microscope slide, and then we added a drop of 3% hydrogen peroxide to the colony, the ability of the bacteria to produce the catalase enzyme was determined by this assay, appearance of bubbles was taken as catalase positive (Varghese and Mallya 2015).

Methyl red (MR) test: is a test used to determine if enough acid was produced during glucose fermentation, some bacteria may ferment glucose into a stable acid, such as lactic acid, acetic acid, or formic acid, after 24 hours of incubation at 37 $°C$ add 1 to 2 drops of methyl red reagent to an aliquot of MR broth containing bacteria that have been examined, look for a bright red color to form on the surface of the medium right away to signal a successful test; a yellow color change means the test result was negative (Ayed *et al*. 2011).

Vogues-proskauer test: The test tubes of the VP-MR medium were inoculated with 24-hour bacterial isolates and incubated at 37$°C$ for 18h then add 6 drops of potassium hydroxide KOH solution at a concentration 40% of two drops of the α-Naphthol solution 5% were gently shaken and incubated in a 30$°C$ water bath, for 14 minutes, the color of the medium changes from yellow to pink, indicating to positive result (Thille 2016).

Citrate utilization test: Bacteria were tested by streaking them onto a plate of Simmon's citrate agar with a sterile loop and incubating the plate at 37$°C$ for a day, positive results are indicated when the green bromothymol blue in the medium is converted to a blue color by the bacteria that have grown on it throughout the incubation time; negative results are indicated when the medium retains its original green color, (MacWilliams *et al.* 2009).

H2S production test: For the purpose of isolating gram-negative bacteria, the fermentation of carbohydrates and the formation of hydrogen sulfide H2S are measured on a plate of triple sugar iron agar, for this experiment, we inoculated a plate of triple sugar iron agar with the test bacteria by stabbing the needle carrying the pure culture into the bottom of a TSI tube, and then streaking the bacteria onto the medium with a sterile loop, the plate was then incubated at 37$°C$ for 24 hours, the end result is favorable for the fermentation of carbohydrates and detrimental to the generation of hydrogen sulfide (Ditrói *et al*. 2019).

Urease production test: After 24 hours of incubation at 37°C,the urea agar slants were read for a positive result if they were pink and a negative result if they were yellow, analyzing urea and producing ammonia and carbon dioxide are three metabolic tasks for which bacteria were tested in this experiment (Achal and Pan 2011).

3.2.5 Antibiotic susceptibility tests

Disk diffusion method was followed as described by CLSI 2020, to carry out the antibiotics susceptibility test for 16 different antibiotics bacterial suspension, was made by transferring 1-2 bacterial colonies from the original culture into a test tube containing 4 mL of normal saline; this yielded a suspension with a turbidity that was moderate in comparison to the standard turbidity solution previously prepared as in section, and it containe d roughly $1.5x10^8$ CFU/mL, for this experiment, we used a sterile cotton swab to transfer a bacterial suspension to Mueller-Hinton agar medium, and then we incubated the plate for 10 min, the antimicrobial discs were then pressed firmly onto the agar using sterile forceps, the plates were turned over and incubated at 37 °C for 18 to 24 hours, the discs' surrounding inhibition zones were measured in millimeters (mm) in accordance with CLSI guidelines (CLSI 2020). By comparing the isolate's inhibition zone to the illustrated norms for that drug, we were able to determine whether the isolate was susceptible, intermediate, or resistant to that drug in Table 3.8.

Table 3.8 Zone diameter interpretation standards (CLSI- 2020)

No.	Antimicrobial agent	Disc potency (µg/disc)	Diameter of zone inhibition (mm)		
			Sensitive	Intermediate	Resistant
1.	Amikacin	30	≥ 17	15-16	≤ 14
2.	Ampicillin-Sulbactam	10/10	≥ 15	12-14	≤ 11
3.	Cefepime	30	≥ 18	15-17	≤ 14
4.	Cefotaxime	30	≥ 23	15-17	≤ 14
5.	Ceftriaxone	30	≥ 21	14-20	≤ 13
6.	Ceftazidime	30	≥ 18	15-17	≤ 14
7.	Colistin	10	≥ 19	16-18	≤ 15
8.	Ciprofloxacin	5	≥ 21	16-20	≤ 15
9.	Gentamicin	10	≥ 15	13-14	≤ 10
10	Imipenem	10	≥22	19-21	≤ 18
11	Levofloxacin	5	≥ 17	14-16	≤ 13
12	Meropenem	10	≥ 18	15-17	≤ 14
13	Piperacillin	100	≥ 21	18-20	≤ 17
14	Tetracycline	30	≥ 15	12-14	≤ 11
15	Tigecycline	15	≥ 15	12-14	≤ 11
16	Trimethoprim	23.75	≥ 16	11-15	≤ 10

Broth microdilution method used for determined Minimum inhibitory concentration (MIC) according to CLSI-2020 recommendations for MIC break points. Using two types of antibiotic for isolate of *A.baumannii* and there isolate were selected according to sensitivity to there antibiotics and positive to (*OXa*-51 and *OXa*-23) gene.

The procedure of this method was determined according to as the following: Single colony of *A.baumannii* was picked and activated on 10 µL of brain heart infustion without addition of antibiotic after incubation for 24 hr at 37°C. By using a plastic loop, a small touch of the bacterial cell was transferred into 5ml of normal saline, and the number of the cell was adjusted into 1x 10^8 CFU/ml equal to 0.5 McFarland. 10 µl of bacterial cell was transferred into 90µl of muller hinton broth. Add 100 µl antibiotics in well. The concentration of the antibiotic was diluted using two fold reducing steps (1024-1µl), a series of dilution that reduce the original concentration to half by subsequent addition and removing from one well to another. Each well was added 5 µl of 1x10^6cfu/mL bacterial inoculum except one well for negative control the addition mixed and incubated for 18 hr at 37 $°C$. 30 µL of resazurin indicator dye was added to each well, and the plates were put back into the incubator for another four hours at 37℃. The result was read it according to the color change. A minimum inhibitory concentration (MIC) for an antibiotic was determined.

3.2.6 Modified hodge test (MHT) and carbacineto NP test

In the study was used Modified hodge test (MHT) and CarbAcineto NP for phenotypic and genotypic detection of carbapenemase, MHT was performed as descried by (Abouelfetouh *et al.* 2019). Aseptic swabs were made onto sterile Müller-Hinton agar plates containing a 1:10 dilution of a 0.5 McFarland suspension of the carbapenem-resistant standard strain of *E. coli*. A disc of meropenem weighing 10 g was aseptically inserted in the middle of the plate. Each *A.baumanii* isolate was streaked in a single, parallel line from the center of the plate to the edge, the commonly used *Klebsiella pneumonia* isolate served as a reference point for comparison, after incubating the plates for 18-24 hours at 37$°C$, we looked for a cloverleaf indentation in the carbapenem disc's inhibitory zone where the test organism and the standard strain met.

In the CarbAcineto NP test, isolate *A.baumannii* from an LB agar plate was taken up from the plate and suspended in 100 mL of 5M NaCl in two Eppendorf tubes (A and B). Besides the 6 mg/mL of imipenem found in Tube B, both Tubes A and B also contained 100 L of a revealing solution. Phenolphthalein red, a pH indicator, and 0.1 mmol/L $ZnSO_4$ were the main components of the illuminating solution. after incubation in tubes A and B for up to 2 hours at 37 °C, the pH of the phenol red solution was adjusted to 7.8 by adding 2 mL of a phenol red solution 0.5% (wt/vol) to 16.6 mL of distilled water, because the hydrolysis of imipenem into a carboxylic derivative causes the pH value to drop, the presence of carbapenemase was discovered in tube B by observing a change in color of the phenol red solution (from red to yellow/orange) (Dortet *et al.* 2014).

3.2.7 Extraction of genomic DNA from *Acinetobacter*

Genomic BYF DNA Extraction Mini Kit was used to extract the DNA according to the protocol of the kit. This kit was designed for the isolation of DNA from various biological samples. DNA was extracted by this kit using bacterial protocol for gram negative bacteria.

Extraction of DNA was done in this study by the following protocol; One mL from *A.baumannii* culture (overnight) added to 2 μL micro centrifuge tube. The culture was centrifuged for 1 min at 13,000 rpm, the supernatant was discarded. Remain about 50 μl supernatant for resuspension, and then resuspend. The bacterial pellet was re-suspended into remnant supernatant (50μl) by tapping or vigorously vortexing. A volume of 300 μL Buffer MG, 20 μl Proteinase K, and 5 μl RNase A Solution were added into sample tube, and resuspend by vortex vigorously. The lysate was incubated for 15 min at 37 $°C$. After lysis completely add 250 μl Buffer MB to the lysate, and mix by pipetting. add 250 μl 80% ethanol to the lysate and mix by pipetting. Pipette 750 μl of the mixture added into the spin column inserted in a 2.0 μl collection tube, then Centrifugedfor 1 min at 13,000 rpm (RT), and discard the flow-through and collection tube altogether. The spin column was placed into a new 2.0 μl collection tube (additionally supplied), then added 700 μl Buffer MW to the spin column, and centrifuged for 1 min at 13,000 rpm, the flow-through was discarded, and re centrifuged for additionally 1 min to dry the membrane. The pre-lysate was centrifuge for 1 min at 13,000 rpm at room temperature. The supernatant was discoverd ensuring that all liquid is completely removed. And then resuspend by vortexing or tapping of cell pellet to pre-lysis cell perfectly. The spin column was placed into a new 1.5 μl tube, and 50 ~ 100 μl Buffer ME was add directly onto the membrane. Incubate for 1 min at room temperature, and then centrifuge for 1 min at 13,000 rpm to elute.

3.2.8 Assessment of purity and concentration of genomic DNA

The DNA purity and quantity was estimated using Nanodrop spectrophotometer in this study. An amount of 2 μL of each sample was used to estimate DNA concentration and purity by using Nanodrop spectrophotometer show in Figure 3.1.

Figure 3.1 NanoDrop spectrophotometer

3.2.9 Molecular detection of some genes by using PCR

All primers were utilized in this study, synthesized by integrated DNA technologies (IDT) company/Canada as lyophilized product of diverse picomols concentrations. And were milted in sterile deionized water (DW) to obtain a find concentration of 100 pmol/ml of suspension and then diluted to 10 pmo/ml as a working solution.

When amplifying PCR reactions, we employed a thermal cycler made by Labnet/USA. This is the reactant blend: 1x mastermix from (promega/USA) containing MgCl2, dNTPs, Taq DNA polymerase, reaction buffer, and green and yellow dyes to monitor electrophoresis progress showing in Table 3.9

Table 3.9 The components of amplification reaction for all genes used in this study

Components	Volume / reaction
PCR master mix (1x)	10
Primer forward (10pmol/Ml)	1
Primer reverse (10pmol/ML)	1
Template DNA	2
Nuclease free water	6
Total reaction volume	20

All amplification reactions were done carrying on ice at a septic condition inside a laminar air flow cabinet. Negative control was used that contained all components except DNA template to detect any contaminating DNA in the reaction. The program which were adopted in PCR analysis for all genes are show in Table 3.10

Table 3.10 Program which were adopted in PCR analysis for all genes

No.	Phase	Tm (°C)	Time	No. of cycle
1	Initial Denaturation	94°C	5 min.	1 cycle
2	Denaturation -2	94°C	45sec.	35 cycle
3	Annealing	55°C	45sec.	
4	Extension-1	72°C	45sec.	
5	Extension -2	72°C	10 min.	1 cycle

Agarose gel was prepared by dissolving 1.5 g of agarose in 100µl of TBE (1X) (pH 8) by using boiling water bath. Left to cool to 50º C and 5 µl of 0.5 mg / ml of Ethidium bromid was added and mixed well. We fixed the comb on one end of the tray and poured off the agrose gently to avoid the formation of any bubbles. Left it to solidify at room temperature, then removed the comb gently from the tray and fixed the tray in an electrophoresis chamber, we loaded the DNA sample (50 µl) in the well and one of the wells was filled with DNA ladder (5 µl of DNA ladder mixed with 1 µl of loading buffer) fill the electrophoresis chamber with TBE buffer (1X). The electrophoresis ran for 80 minutes at 70 Volts. DNA bands were seen with the help of a UV-transilluminator, and photographs of gels were taken using a digital camera appear in Figure 3.2 .

Figure 3.2 Gel electrophoresis

3.2.10 Statistical analysis

The frequency and percentage has been calculated by using the IBM SPSS version 28.0 for non-parametric data and was subjected to one-way analysis of variance (ANOVA) in the package program. Groups differences were determined according to $p < 0.05$ significance level.

4. RESULTS AND DISCUSSION

A. baumannii was detected in 64 (53.3%) of a total of 120 burn, wound, blood, urine, sputum samples with morphological, biochemical and molecular tests. The distribution of investigated clinical specimens 120 was shown in Table 4.1. Antibiotic susceptibility testing using 16 antibiotics discs to *A. baumannii* isolates were obtained. Multi-drug resistance was determined in all *A. baumannii* isolates, the results of the polymerase chain reaction (PCR) showed that 50 of the isolates produced by this investigation carried the some carbapenemases gene (*bla*OXA-51, *bla*OXA-23, *bla*OXA-24, *bla*OXA-58, *bla*OXA-143, *bla*OXA-40, and ISA-BAL).

Table 4.1 Number of hospital specimens

Hospitals	Number of specimens
Al-Kindi Hospital	26 (31%)
Al-Yarmouk Hospital	21(25%)
Al- kadhimiya General Hospital	25(30%)
AlKarama hospital	28(34%)
Total	120 (100%)

4.1 Identification of *A. baumannii* and Antibiotic Resistance/Susceptibility

A total of 120 clinical specimens were cultured on MacConkey agar, selective medium Chrom agar *Acinetobacter* base and also on Chrom agar col-APSE base plates at $37°C$ for 24 hours incubation, the isolates of *A. baumannii* colonies appeared small, pale, lactose non-fermenter colonies on MacCkonkey agar medium and showed Figure 4.1A. *A.baumannii* colonies appeared cream, opaque on Chrom agar col-APSE and showing in 4.1B. *A. baumannii* appeared salmon-red and bright colonies on Chrom agar *Acinetobacter* base medium and showed in Figure 4.1 C. Gram's stain was used to isolate and identify the bacteria. The *A.baumannii* colonies consisted of Gram-negative, semi-spherical bacilli.

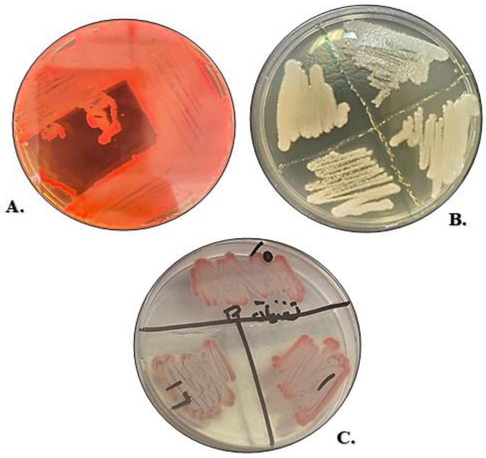

Figure 4.1 *A.baumannii* on A. MacCkonkey agar, B. Chrom agar col-APSE, C. Chrom agar *Acinetobacter*

Some biochemical tests were performed for more validation results as showed in Table 4.2. Biochemical tests have been used to confirm the diagnosis of *A. baumannii*. As a result of the biochemical tests, all of the 64 colonies determined as suspicious out of 120 samples were positive in the catalase test, Citrate Utilization and Simmons Citrate test and Urease test, while the results of the oxidase test, Indole test, Triple- Sugar Iron Agar H2S, Methyl Red and Vogues- Proskauer test were determined as negative. And in the Motility test was determined as Non-mobile, the results of the tests performed for the identification of *A. baumannii* are given in Table 4.2. The images obtained during the tests are given in Figure 4.2 and Figure 4.3 According to the morpholigical and biochemical test results in this study, 64 *A.baumannii* were identificated in 120 samples.

Table 4.2 Biochemical test results of *A.baumannii* Isolates

Biochemical test	Results
Oxidase	-
Catalase	+
Indole	-
Urease	+
Triple- Sugar Iron Agar H2S	-
Methyl Red	-
Citrate Utilization and Simmons Citrate	+
Vogues- Proskauer	-
Motility	Non-motility

+: Positive result, -: Negative result

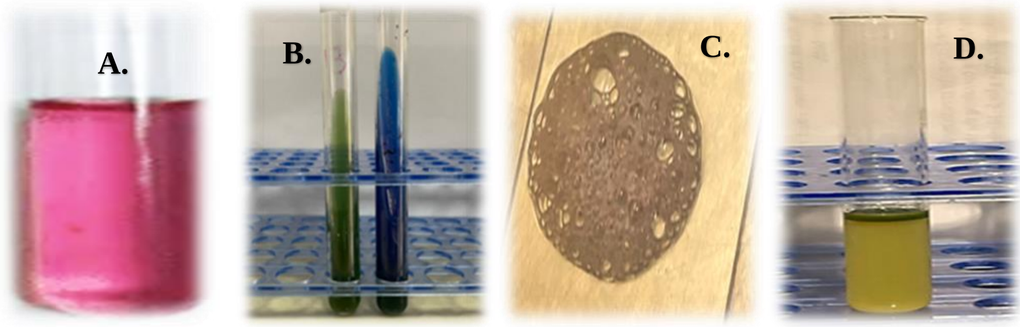

Figure 4.2 A. Urease test, B:Simmon citrate test, C.Catalase test and D. Indole test for *A.baumannii*

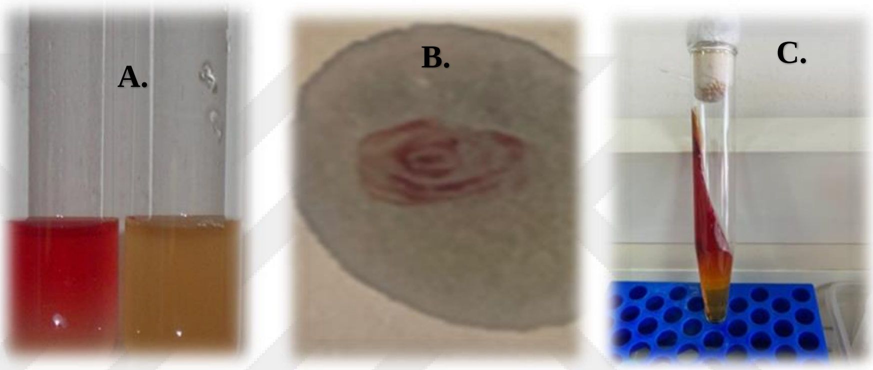

Figure 4.3 A. Methel Red test, B. Oxidase test, C. TSI test for *A. baumannii*

Distributions of *A. baumannii* among clinical isolates were given in Figure 4.4 and *A. baumannii* isolate rates in clinical samples showed in Table 4.3.

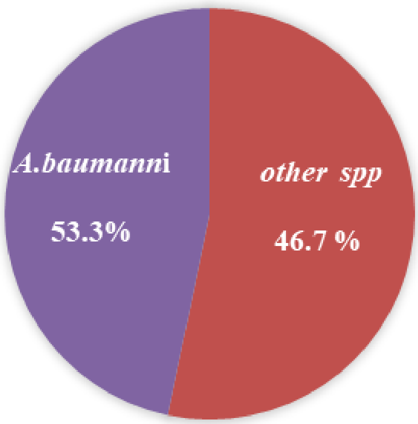

Figure 4.4 Distribution of *A. baumannii* among 120 clinical isolates

Table 4.3 *A. baumannii* isolate rates in clinical samples

Specimens source	**Samples (%)**	**Number and percentage of *A. baumannii***
Wound infections	26 (21.6%)	13(20.3%)
Burns swab	44 (36.6%)	25(39%)
Sputum swab	20 (16.6%)	10(15.6%)
Blood	20 (16.6%)	12(18.7%)
Urine	10 (8.4%)	4 (6.3%)
Total	120 (100%)	64 (100%)

The susceptibility test of all 64 (53.3%) *A.baumannii* was determined by using the disk diffusion method, this test was conducted on 16 antibiogram discs. These bacteria were resistant to 16 antibiotics in about Cefotaxime 100%, Cefepime 98.8%, Piperacillin 96% and Ciprofloxacin 96%, Ceftriaxone and Gentamycin 85.9% and Tigecycline 84.4%, Levofloxacin 75.0%, Imipenem 95.3% , Meropenem 93.8% and Amikacin 71.9% whereas, Tetracycline 48.4%, Colistin 43.8%,Trimethoprim 76.6% , Ampicillin 40.6%. Consequently, those percentages show the higher resistance with Cefotaxime 100% and the lowest resistance with Ampicillin (40.6%). As showed in Figure 4.5.

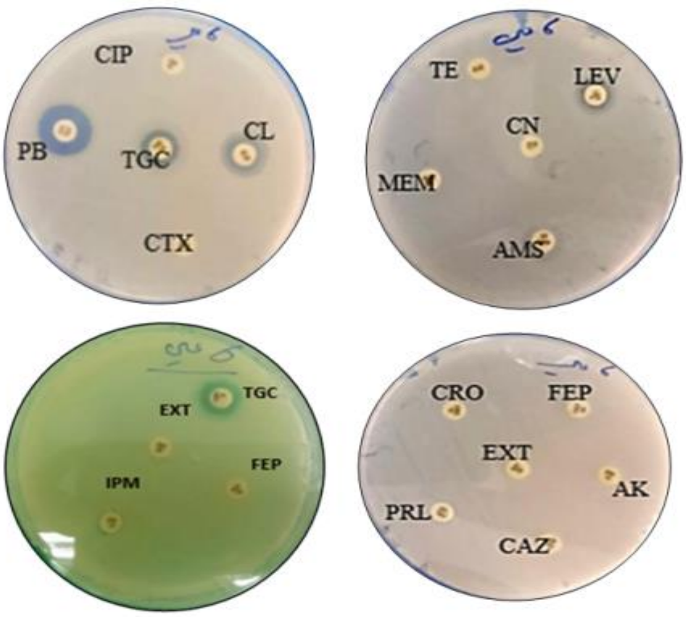

Figure 4.5 Disc diffusion test

The results of antibiotics susceptibility for *A. baumannii* amoung 64 isolates. We find 60 isolates have resistance both iminipem and meropenem. This isolates have carbapenemase enzyme according to results. We selected 50 isolates for phenotyping and molecular detection were shown in Table 4.4

Table 4.4 Rate of antibiotics susceptibility for 64 *A.baumannii*

No	Antibiotics	Dose (µg)	Percentage (%)		
			R	**I**	**S**
1.	Amikacin	30	46 (%71.9)	6 (%9.4)	12 (%18.8)
2.	Ampicillin/ Sulbactam	10/10	25 (%40.6)	14 (%20.3)	25 (%39.1)
3.	Cefepime	30	63 (%98.4)	0 (%0.0)	1 (%1.6)
4.	Cefotaxime	30	64 (%100.0)	0 (%0.0)	0 (%0.0)
5.	Ceftazidime	30	61 (%95.3)	2 (%3.1)	1 (%1.6)
6.	Ceftriaxone	30	61 (%95.6)	3 (%4.7)	0 (%0.0)
7.	Ciprofloxacin	5	62 (%96.9)	1 (%1.6)	1 (%1.6)
8.	Colistin	10	28 (%43.8)	16 (%25.0)	20 (%31.3)
9.	Gentamycin	10	55 (%85.9)	6 (%9.4)	3 (%4.7)
10.	Imipenem	10	61 (%95.3)	2 (%3.1)	1 (%1.6)
11.	Levofloxacin	5	48 (%75.0)	13 (%20.3)	3 (%4.7)
12.	Meropenem	10	60 (%93.8)	1 (%1.6)	3 (%4.7)
13.	Piperacillin	100	62 (%96.8)	1 (%1.6)	1 (%1.6)
14.	Tetracycline	30	31 (%48.4)	17 (%26.6)	16 (%25.0)
15.	Tigecycline	15	54 (%84.4)	4 (%6.3)	6 (%9.4)
16.	Trimethoprim	23.75	49 (%76.6)	5 (%7.8)	10 (%15.6)

R: resistence I: intermedite S: sensitive

Broth microdilution method in the study to determine the minimum concentration of levofloxacin and amikacin that able to inhibit bacterial growth. MIC values of amikacin and levofloxacin against isolates of *A.baumannii* (isolate 1 and 2) were showed in Figure 4.6. The concentrations of antibiotics are between (2µl, 4µl, 8 µl, 16µl, 32µl, 64µl, 128µl, 256µl, 512µl) and 0 is negative control.

It was determined that the MIC value of levofloxacin was 8 µl for isolate 1 and 2 µl for isolated 2. This test results showed in Figure 4.6C. It was determined that the MIC value of amikacin was 512 µl for isolate 1 and 16µl for isolate 2. This test results showed in Figure 4.6D.

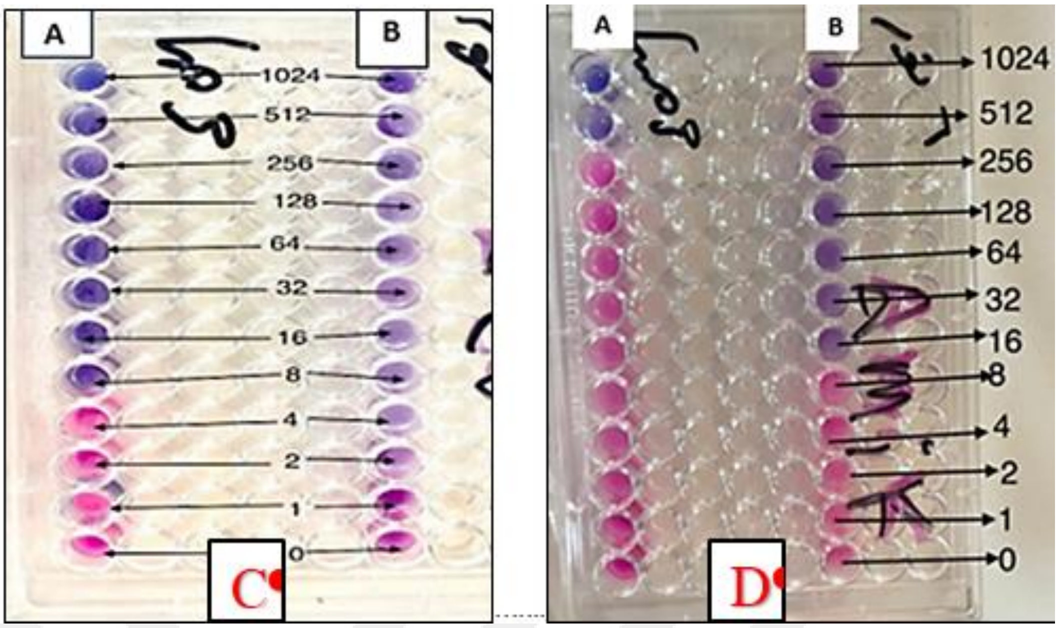

Figure 4.6 C. MIC test for levofloxacin against *A. baumannii* D. MIC test for Amikacin against *A. baumannii*

4.2 Modified Hodge test (MHT) and Carbapenems NP test

Fifty carbapenem-resistant isolates were screened for carbapenemase production by phenotypic method (MHT) compared with *E.coli ATCC* as a control and *Klebsiella pneumonia*.

The 50 *A. baumannii* isolates that showings detect as resistance against meropenem and iminepem were used in MHT test. The MHT test present 25 of *A. baumannii* isolates showed positive result. However, none of the other 25 *A. baumannii* isolates were positive for carbapenemase production.

Bacteriostatic zone around the meropenem disk was used to report the presence or absence of carbapenemase generating isolates. Positive results were recorded when the bacteriostatic zone diameter was less than 6 mm against E.Coli ATCC, while negative results were reported when the zone diameter was greater than 6 mm,which shown in Figure 4.7.

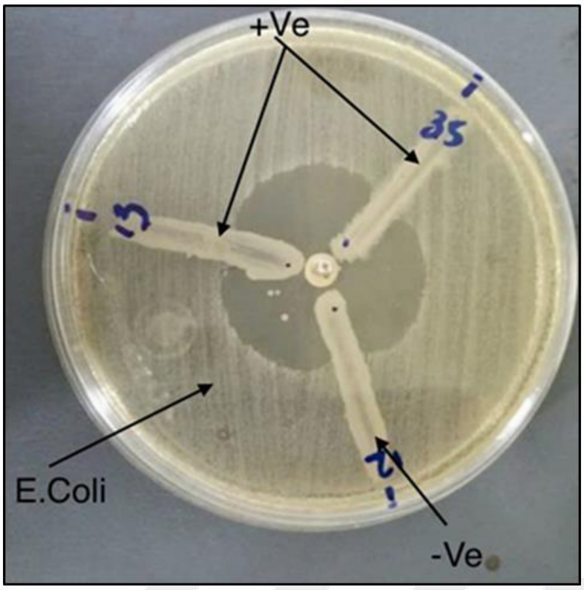

Figure 4.7 MHT test for *A.baumannii*, *Positive:+Ve, Negative:-Ve*

In the same time with MHT test fifty carbapenem-resistant isolates were screened for carbapenemase production by phenotypic method Carbapenems NP test. The studied carbapenem-resistant *A. baumannii*, 20 isolates were positive by Carba NP. The other 30 isolates give negative results in Carba NP test. Results were given in Table 4.8.

4.3 Molecular Study of *A.baumannii*

4.3.1 Prutiy of extraction DNA

The NanoDrop-1000 technique and PCR analysis proved to be beneficial in the measurement of both quantity and purity of DNA in the samples (Bunu *et al*. 2020). All samples were determined purity of DNA value as shown in Table 4.5.

Table 4.5 The purity of DNA measured by Nanodrop spectrophotometer

No.	Nucleic acid conc. (ng/mL)	260/280 purity	No.	Nucleic acid conc. (ng/mL)	260/280 purity
1	118	1.8	26	123	1.8
2	158	1.69	27	165	1.9
3	192.9	1. 87	28	123	1.8
4	155	1.71	29	130	2
5	76	1.9	30	117	2
6	176	1.85	31	55	1.8
7	166	2.00	32	77	1.9
8	76	1.65	33	167	1.98
9	223	2	34	145	1.87
10	115	1.79	35	172	1.9
11	98	1.9	36	70	1.7
12	278	1.83	37	150	1.9
13	123	1.83	38	77	1.8
14	125	1.78	39	79	1.69
15	110	1.75	40	161	2.03
16	270	1.75	41	123	1.7
17	77	1.9	42	88.8	1.8
18	56	1.8	43	66	1.86
19	60	1.69	44	197	1.9
20	163	2	45	150	1.9
21	224	1.8	46	77	1.8
22	99.9	1.9	47	90	1.54
23	77	1.88	48	143	1.9
24	197	1.9	49	176	1.79
25	222	1.7	50	189	1.8

4.3.2 Identification of *A. baumannii* by PCR technique

PCR was used to investigate the presence of the 16s rRNA gene in *A. baumannii* isolates. As a result of the analysis, it was determined that 64 isolates carried the 16s rRNA gene with a length of 585 bp. Thus, similar results were obtained with the convensional identifaciton (culture and biochemical test) method. The some PCR image is given in Figure 4.8.

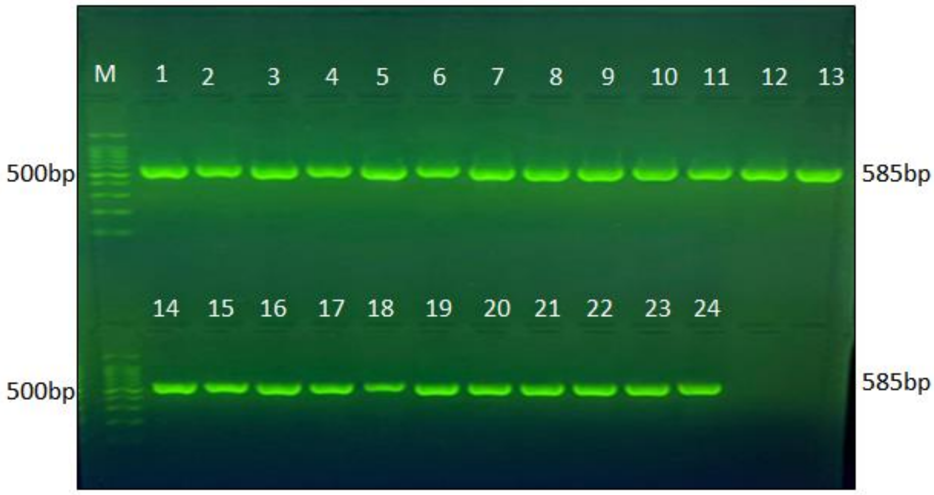

Figure 4.8 PCR product for 16s rRNA gene

4.3.3 Detection of carbapenemase genes by PCR

In this study, 60 *A.baumannii* with carbapenemases was determined phenotypically. In order to detection the presence of carbapenemase genes *bla*OXA-143 band size 150 bp, *bla*OXA-58 band size 599bp, *bla*OXA-51 band size 353 bp, *bla*OXA-40 band size 246 bp, *bla*OXA-24 band size 828 bp, *bla*OXA-23 band size 501 bp and ISA-BAL band size 390 bp among *A. baumannii* clinical isolates with PCR. The PCR reaction included 50 carbapenemase-producing isolates for the detection of the sets of carbapenemase genes. Analysis of the bands on gel electrophoresis and molecular weight comparison with a 100 bp DNA ladder have confirmed the PCR results. The carbapenemase gene PCR results from *A. baumannii* clinical isolates showing in the Figure 4.9 .

In this study we found the OXA-58 gene in 2 (%4.0), isolates, *bla*OXA-51 gene in all (%100), isolates OXA-40 gene in 12 (%24) isolates, OXA-24 gene in 14 (%28) isolates, OXA-23 gene in 36 (%78.1) isolates, ISA-BAL gene in 46 (%90) isolates and no find OXA-143 gene from 50 *A. baumannii* isolate by PCR. These results were given in Table 4.6.

Table 4.6 Results of carbapenemase genes detected in *A. baumannii* isolates

Genes	Positive	Negative	Total
OXA-51	50 (%100.0)	0 (%0.0)	50 (%100.0)
OXA-23	36 (%78.1)	14 (%21.9)	50 (%100.0)
OXA-24	14 (%28.0)	36 (%72.0)	50 (%100.0)
ISA	46 (%90.0)	4 (%8.0)	50 (%100.0)
OXA-40	12 (%24.0)	38 (%76.0)	50 (%100.0)
OXA-143	0 (%0.0)	50 (%100.0)	50 (%100.0)
OXA-58	2 (%4.0)	48 (%96.0)	50 (%100.0)

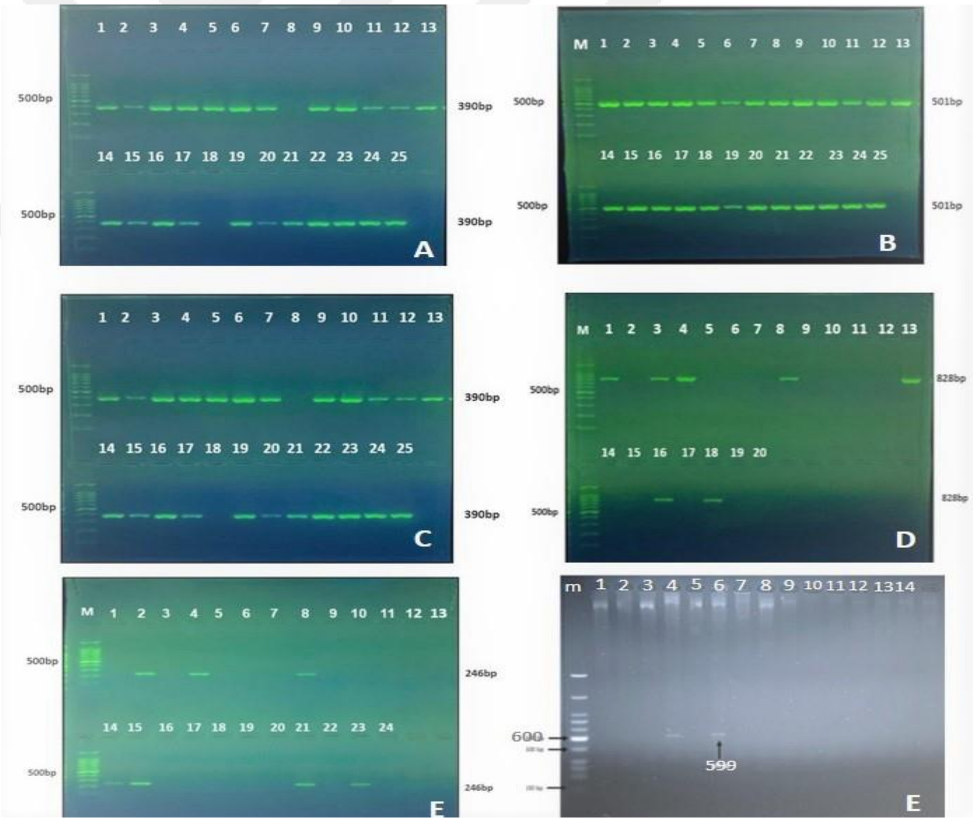

Figure 4.9 A.blaOXA51 gene band, B. blaOXA23 gene band, C.ISA-BA1 gene band, D.blaOXA-24 gene band, E. blaOXA-40 gene band and F. blaOXA-58 gene band.

4.3.4 Comparisons of phenotyping and genotyping results for carpapenemase in *A.baumannii*

Phenotyping and genotyping results were compared. In the study were determined 50 isolates (100%) positive result by disc diffusion, phenotyping showing 24 (48%) isolates positive of MHT , 20(40%) isolated positive CarbAcineto NP. The genotyping detected negative result on blaOXA-143 gene, and 2 isolates positive result on *bla*OXA-58, the 50 (100%) isolates positive result on *bla*OXA-51and *bla*OXA40 showing 12 (23%) isolates positive but *bla*OXA-24 showing 14 (28%) positive result, and *bla*OXA-23 showing 36 (72%) isolated positive, the last gene ISA-BAL the result appear 46 (92%) isolated positive and these results are given in the Table 4.7.

Table 4.7 Comparisons of phenotyping and genotyping carbapenemase results in *A.baumannii isolates.*

No	DDT	MHT	CANP	b-51	b-23	b -24	ISA	b-143	b-40	b-58
1	+	-	-	+	+	-	+	-	-	-
2	+	-	+	+	+	-	+	-	+	-
3	+	-	+	+	+	-	+	-	+	-
4	+	+	+	+	-	-	+	-	-	-
5	+	+	+	+	+	+	-	-	-	-
6	+	+	-	+	+	-	+	-	-	-
7	+	-	-	+	+	-	+	-	-	+
8	+	-	+	+	+	-	+	-	+	-
9	+	+	-	+	-	-	+	-	-	-
10	+	+	-	+	+	-	+	-	-	-
11	+	-	-	+	+	-	+	-	-	-
12	+	+	-	+	-	-	+	-	-	-
13	+	-	+	+	-	+	+	-	-	-
14	+	+	+	+	+	+	+	-	+	-
15	+	-	-	+	+	+	+	-	-	-
16	+	+	+	+	+	+	+	-	+	-
17	+	+	+	+	-	+	+	-	-	+
18	+	+	-	+	+	+	+	-	-	-
19	+	-	+	+	+	-	-	-	+	-
20	+	+	-	+	+	-	+	-	-	-
21	+	+	-	+	+	-	+	-	-	-
22	+	+	-	+	+	-	+	-	-	-
23	+	-	-	+	+	-	+	-	-	-
24	+	-	-	+	+	-	+	-	-	-
25	+	+	-	+	-	-	+	-	-	-
26	+	+	+	+	+	+	+	-	-	-

Table 4.7 Comparisons of phenotyping and genotyping carbapenemase results in *A.baumannii isolates* (Continue)

27	+	-	-	+	-	-	+	-	-	-
28	+	+	+	+	+	+	+	-	+	-
29	+	+	-	+	+	+	+	-	-	-
30	+	-	+	+	+	-	+	-	+	-
31	+	-	-	+	+	-	+	-	-	-
32	+	-	-	+	-	-	+	-	-	-
33	+	+	+	+	+	+	-	-	-	-
34	+	-	+	+	+	-	+	-	-	-
35	+	+	-	+	-	-	+	-	-	-
36	+	+	-	+	+	-	+	-	-	-
37	+	-	-	+	+	-	+	-	-	-
38	+	+	+	+	-	+	+	-	-	-
39	+	-	-	+	+	-	+	-	-	-
40	+	-	+	+	+	-	+	-	+	-
41	+	-	+	+	-	+	+	-	+	-
42	+	-	-	+	+	-	+	-	-	-
43	+	+	-	+	+	+	-	-	-	-
44	+	-	-	+	-	-	+	-	-	-
45	+	-	-	+	+	-	+	-	-	-
46	+	-	-	+	+	-	+	-	-	-
47	+	+	+	+	-	-	+	-	+	-
48	+	+	-	+	+	-	+	-	-	-
49	+	-	+	+	-	-	+	-	+	-
50	+	-	-	+	+	-	+	-	-	-

DDT: Meropenem and iminepem resistance isolate by disc diffusion**, MHT:** modified hodge test, **CANP:** CarbAcineto NP, **b**:blaOXA gene, **ISA**: ISA-BAL gene

Interestingly, demonstrated statistically significant (p-value<0.05) positive correlations with the same isolates, namely, the count of genes detected in combination. On the other hand, statistically significant negative correlations were obtained between certain isolates and the the carbapenem genes.

The false-negative results missed the MHT test were linked to *bla*-OXA 143 (0/50), *bla*-OXA 58 (25/50), and *bla*-OXA 51 (0/50),and *bla*-OXA 40 (18/50) , and *bla*-OXA 24 (23/50),) and *bla* OXA 23 (6/50), and ISA-baI (1/50). These results were given in Table 4.8.

Table 4.8 Comparison of MTH test and PCR results

Genes		MTH No. (%)		Probability
		Positive	**Negative**	
OXA-51	Positive	24 (100.0)	26 (100.0)	-
	Negative	0 (0.0)	0 (0.0)	
blaOXA23	Positive	16 (66.7)	20 (76.9)	0.420
	Negative	8 (33.3)	6 (23.1)	
blaOXA24	Positive	11 (45.8)	3 (11.5)	0.007
	Negative	13 (54.2)	23 (88.5)	
ISAbaI	Positive	21 (87.5)	25 (96.2)	0.260
	Negative	3 (12.5)	1 (3.8)	
OXA143	Positive	24 (100.0)	26 (100.0)	-
	Negative	0 (0.0)	0 (0.0)	
OXA40	Positive	4 (16.7)	8 (30.8)	0.243
	Negative	20 (83.3)	18 (69.2)	
OXA58	Positive	1 (4.2)	1 (3.8)	0.954
	Negative	23 (95.8)	25 (96.2)	

$P < 0.05$ shows the significant in the parameters.

The false-negative results missed by the Carba NP test were linked to *bla*-OXA 24 (26/50), *bla*-OXA 143 (0/50) and *bla*-OXA 40 (30/50), and ISA-baI (1/50) and ISA-baI (1/50), and bla OXA 51 (0/50) and bla OXA 23 (7/50) and blaOXA 58 (29/50). These results given in Table 4.9.

Table 4.9 Comparision of CarbAcineto NP test and PCR results

Genes		CarbAcineto NP No. (%)		Probability
		Positive	**Negative**	
OXA-51	Positive	20 (100.0)	30 (100.0)	-
	Negative	0 (0.0)	0 (0.0)	
blaOXA23	Positive	13 (65.0)	23 (76.7)	0.368
	Negative	7 (35.0)	7 (23.3)	
blaOXA24	Positive	10 (50.0)	4 (13.3)	0.005
	Negative	10 (50.0)	26 (86.7)	
ISAbaI	Positive	17 (85.0)	29 (96.7)	0.136
	Negative	3 (15.0)	1 (3.3)	
OXA143	Positive	20 (100.0)	30 (100.0)	-
	Negative	0 (0.0)	0 (0.0)	
OXA40	Positive	12 (60.0)	0 (0.0)	0.000001
	Negative	8 (40.0)	30 (100.0)	
OXA58	Positive	1 (5.0)	1 (3.3)	0.768
	Negative	19 (95.0)	29 (96.7)	

$P < 0.05$ shows the significant in the parameters.

The World Health Organization has indicated that *A. baumannii* a priority antibiotic-resistant pathogen that poses the greatest threat to human health (WHO 2017). Carbapenem-resistant pneumonia, as well as wound, bloodstream, and urinary tract infections, is caused by *Acinetobacter*, these infections frequently affect ICU patients, Carbapenem-resistant it is possible for *Acinetobacter* to harbor mobile genetic elements that are freely transferred across bacteria (Pogue *et al.* 2013).

According to the morphological, biochemical and molecular test (16rRNA for *A.baumannii*) results in this study, were identificate 64 *A.baumannii* in 120 clinic samples. The result presented in the current study coincide with the result recorded by Qader (2021), according to conventional methods (relying on microscopic and cultured inspection and biochemical test) and molecular approach employing 16SrRNA gene, only 75 isolates were belong to *A. baumannii* out of 150 bacterial isolates.

The antibiotic resistance/susceptibility test of all 64 *A.baumannii* was determined by using the disk diffusion method. This test was conducted on 16 antibiogram discs. These bacteria were resistant to 16 antibiotics in about cefotaxime 100%, cefepime 98.8%, piperacillin 96% and ciprofloxacin 96%, ceftriaxone and gentamycin 85.9% and tigecycline 84.4%, levofloxacin 75.0% and amikacin 71.9% whereas, tetracycline 48.4%, colistin 43.8%, ampicillin 40.6%. Consequently, those percentages show the higher resistance with Cefotaxime 100% and the lowest resistance with Ampicillin 40.6%, the results of trimethoprim resistance *A. baumannii* was agreed with a study conducted by Angoti *et al*. (2016) that showed *A. baumannii* resist a wide range of antibiotics including Imipenem in percentage ratio (99%) which nearest to the current study result, the percentage of Tetracycline resistance (48. 4%) according to our study was close to the percentage that obtained by Beheshti *et al.* (2020), who shows the percentage of tetracycline resistance was (47.95%), although the current study results closely to Thirapanmethee *et al*. (2020), this data shows that all of the isolates tested positive for resistance to carbapenems; specifically, 100% of the isolates tested positive for resistance to imipenem, followed by 98.91% resistance to meropenem, Additionally, the majority of the isolates showed high resistance rates to ciprofloxacin (97.81%), ceftazidime (96.72%), gentamicin (91.26%), and amikacin (80.87%).

Broth microdilution method in the study to determine the minimum concentration of levofloxacin and amikacin that able to inhibit bacterial growth, It was determined that the MIC value of levofloxacin was 8 µl for isolate 1 and 2 µl for isolated 2, it was determined that the MIC value of amikacin was 512 µl for isolate 1 and 16µl for isolate 2, this result is closely related to the result of the MIC of levofloxacin against *A. baumannii* in the study of Kaviani (2020), who show the MIC of levofloxacin was 4 µl. Also, agreed with the result of Göttig *et al.* (2014) who show the MIC of levofloxacin was 32/100 µl. A study by Vergalli *et al.* (2020) showed the value of MIC of quinolones and doses for early killing (DEK) against gram-negative bacteria. Another study shows the MIC value between (8 µl- >128 µl) of amikacin against different strains of *A. baumannii* (Beganovic *et al.* 2019).

Fifty carbapenem-resistant isolates were screened for carbapenemase production by phenotypic method MHT and Carba NP. 25 (50%) of *A. baumannii* isolates were positive in MHT test and 20 (40 %) isolates were positive by Carba NP. Similar result by Rao *et al*. (2022) demonstrate that 63.7% of *A. baumannii* as carbapenemase producers identified by MHT test, while 63% by CarbAcineto NP test exibiting 94.74% sensitivity, 93.22% specificity when compared to Modified Hodge test. Also, 84% carbapenamase reported by Modified Hodge method (Moulana *et al.* 2020). Norwise, the study of Abouelfetouh *et al.* (2019) 95.94% of *A. baumannii* were positive for carbapenemase by CarbAcineto NP test.

In PCR tests were detected OXA-58 gene in 2 isolates (%4.0), *bla*OXA-51 gene in all isolates (%100), OXA-40 gene in 12 isolates (%24), OXA-24 gene in 14 isolates (%28), OXA-23 gene in 36 isolates (%78.1), ISA-BAL gene in 46 isolates (%90) and no find OXA-143 gene from 50 *A. baumannii* isolate.

Iraqi study showed the results of PCR showed that 75 samples were confirmed to be *A. baumannii* through polymerase chain reaction (PCR), with the genus-specific *bla*OXA-51 primer yielding a 353 bp amplification band (Qader 2021). Otherwise, 11 isolates from 183 were detected by Thirapanmethee *et al.* (2020) carbapenemase resistance according to the *bla*-oxa-51-like gene, an intrinsic enzyme marker was detected in all clinical isolates, *bla*-oxa-23-like gene was presented in the most of isolates (68.31%), *bla*-oxa-40/24-like gene (4.92%) and *bla*-oxa-58-like gene (1.09%), also, they demonstrate all isolates were resistant to carbapenems, with 50 % resistance to imipenem, followed by meropenem (98.91%).

Phenotyping and genotyping results were compared in the study, in the were determined 60 isolates (100%) positive result by disc diffusion, phenotyping showing 24/50 (48%) isolates positive of MHT , 20/50 (40%) isolated positive CarbAcineto NP, the genotyping detected negative result on *bla*OXA-143 gene, and 2 isolates positive result on *bla*OXA-58, the 50 (100%) isolates positive result on *bla*OXA-51and *bla*OXA40 showing 12 (23%) isolates positive but *bla*OXA-24 showing 14 (28%) positive result , and *bla*OXA-23 showing 36 (72%) isolated positive , the last gene ISA-BAL the result appear 46 (92%) isolated positive, the false-negative results missed by the Carba NP test were linked to *bla*-OXA 24 (26/50), *bla*-OXA 143 and *bla*-OXA 40 (30/50), and ISA-baI (1/50) carbapenemases, the false-negative results missed the MHT test were linked to *bla*-OXA 143 (0/50), *bla*-OXA 58 (25/50), and *bla*-OXA 51 (0/50),and bla-OXA 40 (18/50) , and *bla*-OXA 24 (23/50),) and *bla*-OXA 23 (6/50), and ISA-baI (1/50), and in this study was reported positive result of phenotyping MHT methods showing (48%), Phenotypic detection of carbapenemases has the benefits of being inexpensive, simple to perform, and requiring little to no specialized or costly equipment, but it has low specificity and sensitivity, to compare the efficacy of various diagnostic methods, PCR screening for carbapenem resistance genes and insertion sequences was used as a gold standard. The nearest result found by Fan *et a*l. (2020) showed 83 carbapenem-resistant isolates of *A. baumannii* from a total of 135 non-replicate clinical and 52 carbapenem-sensitive strains in the MHT result test, they present the higher resistance of OXA-51, OXA-23, ISAbal and lower resistant of OXA-58 and OXA-24 against meropenem. Study result is similar to Abouelfetouh *et al*. (2019) result showed 58 *A. baumannii* isolates had a positive result in the MHT test, while the result showed a negative in the remaining 16 isolates.

5. CONCLUSIONS AND RECOMMENDATION

This study attracted attention to *A.baumannii* as one of the important causes of nosocomial infections in our hospitals, especially in wound infections, burns, sputum, blood and urine. CHROMagar *Acinetobacter* and MacCkonkey agar medium gave excellent results for primary and rapid detection of this species with accurate results.

As this investigation shown, most local clinical isolates of *A.baumannii* were resistant to many antibiotics, including the most effective class of antibiotics now in use to treat this type of bacterium in hospitals. Carbapenems, amikacin and levofloxacin were active against most local isolates. Carbapenem-resistant *A. baumannii* (CR-AB) has emerged as a serious threat to the efficacy of treatment protocols due to its widespread discovery.Our study demonstrated the role of 16S rRNA, carbapenems (*bla*OXA-51, *bla*OXA-23, *bla*OXA-24, *bla*OXA-58, *bla*OXA-143, *bla*OXA-40 and ISA-BAL) genes for molecular detection *A.baumannii* at the level of genus and species respectively.*bla*OXA-51 and *bla*OXA-23 genes were the most prevalent gene in the multidrug-resistant local isolates with a high percentage in contrast with most worldwide studies.*bla*OXA-51 and *bla*OXA-23 were the two most common CR-AB clinical isolates in the examined population, followed by *bla*OXA-58. The most conclusive method for finding carbapenemases in CR-AB clinical isolates was genotypic detection using PCR, closely followed by phenotypic testing with MHT and CarbAcineto NP test.

Based on the findings of the present study, after reviewing the many of the previous trials, the following recommendations can be suggested: The local isolates of *A.baumannii* in Iraqi hospitals should be examined for the presence of different types of beta-lactamase genes.studying the other mechanisms of antibiotic resistance and their genes in local isolates of *A.baumannii* such as outer membrane proteins.such as inhibitors of efflux pumps, in order to combat the bacteria's resistance to these drugs.More research is required for a clearer understanding of biofilm formation stages and contributing factors, as well as its interactions with various antibiotic groups.More research is required to identify other Q.S-related genes, biofilm-forming gene profiles,and their connections to MDR and XDR.

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APPENDICES

APPENDIX 1. Ethics committee

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