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Molecular Detection of Some Antibiotic Resistance Genes of *Acinetobacter baumannii* Isolates from Patients in Erbil/KRG-Iraq

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Masters of Science (MSc.) in Biology

By

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This thesis is dedicated to

- Anyone who has loved, respected, and wishes the best for me throughout all of the years of my study.

VI

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List of Abbreviations

Abbreviation	Mean
AAC	Aminoglycoside acetyltransferases
AG	Aminoglycosides
AK	Amikacin
AMEs	Aminoglycoside-modifying enzymes
AMR	Antimicrobial resistance
ANT	Aminoglycoside adenylyltransferase
APH	Aminoglycoside phosphotransferases
AST	Antimicrobial susceptibility test
ATM	Ceftazidime
BHI	Brain Heart Infusion agar
bp	Base pair
CIP	Ciprofloxacin
CLSI	Clinical Laboratory Standard International
COVID-19	Coronavirus disease of 2019
CRAB	Carbapenem-resistant *Acinetobacter baumannii*
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
ESBLs	Extended spectrum B-lactamases
GC	Guanine-cytosine

GEN	Gentamicin
GIsul2	Genomic island sul2
GNI	Gram Negative Identification
H_2O_2	Hydrogen peroxide
ICU	Intensive care units
ID	Identification
IPM	Imipenem
LB	Lauria Bertani broth
LEV	Levofloxacin
M.T.P	Microtiter plate
MAR	Multiple antibiotic resistance
MDR	Multi drug resistant
MEM	Meropenem
MFP	Membrane fusion protein
MIC	Minimum inhibitory concentrations
NET	Netilmicin
OMP	Outer membrane protein
OMPs	Outer membrane proteins
PAR	Pan antibiotic resistant
PCR	Polymerase chain reaction
PIP	Piperacillin
QRDRs	Quinolone resistance-determining regions

QS	Quorum Sensing
RNA	Ribonucleic acid
RND	Resistance-Nodulation Cell-Division
RPPs	Ribosomal protection proteins
SXT	Trimethoprim/sulfamethoxazole
TBE	Tris-Borate-EDTA Buffer
TCSs	Two component systems
TIG	Tigecycline
TSB	Tryptone Soy Broth
UTI	Urinary tract infections
XAR	Extensively antibiotic resistance

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Abstract

Recently, multidrug-resistant *Acinetobacter baumannii* has raised its impact on patient care specifically after covid-19 pandemic. The potential revolution of *Acinetobacter baumannii* is driven by the undetectable numbers of gene resistance that is acquired.

Of 570 patient specimens including blood, sputum, urine, stool, pus, swab, and body fluid, 23 (4.04%) *Acinetobacter baumannii* were identified by biochemical tests and VITEK 2 compact system.

Identification of *Acinetobacter baumannii* has been studied phenotypically by conventional biochemical tests and VITEK 2 compact system, and genotypically by using polymer chain reaction (PCR) to detect 16S-23S rRNA gene intergenic spacer region (ITS) and sequencing the amplicons by Sanger sequence technique. Isolates characterizations included biochemical identification, plasmid detection, quantification of biofilm formation using microtiter plate method, antibiotic resistance profile by using VITEK 2 compact system, and PCR amplification gene resistance. Twenty-two resistance genes were targeted including β-lactamase genes: $bla_{\text{OXA-51}}$, $bla_{\text{OXA-58}}$, $bla_{\text{OXA-23}}$, bla_{TEM}, $bla_{\text{NDM,}}$ and bla_{SHV}, aminoglycoside genes: *aph(3')-VI, aacA4, aadB, strA* and *strB*, sulfonamide genes: *sul I* and *sul II* , multidrug efflux gene *adeB* , tetracycline genes: *tetA* and *tetB*, macrolide genes: *msr(E), mph(E)* and *erm 42*, Trimethoprim gene: *dhfr1* and Fluoroquinolones genes: *parC* and *gyrA*.

Out of 100 (21.28%) who were covid-19 patients, 13 (13%) isolates were *Acinetobacter baumannii*. All *Acinetobacter baumannii* isolates carried a plasmid and there were no strong biofilm formation. morever, 41.2% of the isolates formed medium biofilm which is statistically significant, 35.3% of the isolates formed weak biofilm and 17.6% of the isolates not formed biofilm which are statistically non-significant. Both bacterial chromosome and plasmid carried resistance genes and each isolate with at least seven resistance genes. Besides, this is the first study to display the genetic resistance epidemics in Erbil/Iraq, bla_{TEM} detected the disseminated gene in isolates, and *aadB* was not encoded by isolates. The majority of the resistance genes that were not detected by the isolate chromosome were detected on the plasmid of the isolate. Furthermore, new carbapenemase gene sequences have been identified to be acquired by plasmids from other bacterial genera such as *Klebsiella pneumonia* and *Escherichia coli* which

had coinfected cases with *Acinetobacter baumannii* in the study. The new genes are bla_{NDM}, (*Acinetobacter baumannii* strain carbapenems resistance HK19; accession number: OP572243) and bla_{TEM} (*Acinetobacter baumannii* strain beta-lactam resistance HK22; accession number: OP572244). In addition, a new strain *Stenotrophomones maltophilia* (accession number OP422244) was submitted to GenBank and acquired new beta-lactamase genes from *Acinetobacter baumannii* bla_{NDM} and $bla_{OXA\text{-}23}$ genes under accession numbers OP595162, OP595163 respectively. The isolate's metabolism pathway used was carbohydrates at 93.8% and coumarate at 100%.

The results demonstrated the pattern role of plasmid in *Acinetobacter baumannii* resistance and the alternative metabolic pathway in all strains to survive was coumarate metabolic pathway, although the isolates metabolic differences but all strains used the coumarate pathway to survive.

Chapter One

1. Introduction

Acinetobacter genus is a Gram negative bacteria, coccobacillus shape, belongs to the Moraxellaceae family, and widely distributed in nature (Vázquez-López *et al.*, 2020; Kyriakidis *et al.*, 2021). Species included in genus *Acinetobacter*, are characterized by being strictly aerobic, non-fermenting, non-fastidious, lack flagella, catalase-positive, and oxidase-negative bacteria, with a 39% to 47% content of guanine-cytosine (GC) in their DNA sequence (Vázquez-López *et al.*, 2020; Gedefie *et al.*, 2021). *Acinetobacter* phylogenetics has undergone significant changes, originally described as *Micrococcus*, the designation of *Acinetobacter* only being proposed in the 1950's. Since then, *Acinetobacter* taxonomy has been reclassified and over 50 different species have been identified to date (Morris et al., 2019), which are mostly nonpathogenic environmental organisms. However, among infectious species, *Acinetobacter baumannii* (*A. baumannii*) has been described as the most virulent one followed by *A. calcoaceticus* and *A. lwoffii* (Vázquez-López et al., 2020), *A. nosocomialis* and *A. pittii* (Morris et al., 2019). *A. baumannii* is considered as an opportunistic pathogen causing nosocomial infections or hospital acquired infections in hospitalized patients, particularly in intensive care units (ICU), as well as community-acquired infections (Allen et al., 2020). These infections include pneumonia, bloodstream infection, skin and soft tissue infections, wound infection, meningitis, urinary tract infection and endocarditis (Liu *et al.*, 2017; Qader, 2021). Risk factors for these infections include mechanical ventilation, usage of broad-spectrum antibiotics, ICU stay time and coma (Nie et al., 2020). *A. baumannii* is widespread in clinical environments, surviving as a commensal on the skin or hair of hospital staff and patients (AL-Kadmy et al., 2018). Although it has been found in a variety of environmental samples, the natural habitat of *A. baumannii* is still not known. This opportunistic pathogen has remarkable abilities to endure desiccation and starvation, acquire resistance to different classes of antibiotics, and disseminate in and between medical facilities (Hamidian and Nigro, 2019; Karah, Wai and Uhlin, 2021; Castro-Jaimes *et al.*, 2022), environmental persistence, host-pathogen interactions, immune evasion (Sarshar et al., 2021), form biofilms, resist desiccation and pH extremes. These properties facilitate the persistence of *A. baumannii* in the hospital setting and promote the emergence of outbreaks (Castro-Jaimes et al., 2022). Various contaminated objects have been identified that serve as potential reservoirs for this nosocomial pathogen. Human utility articles,

importantly computers, mouse and gloves, as well as pets, have been suggested as causes for the spread of *A. baumannii* in humans (AL-Kadmy et al., 2018). *A. baumannii* isolates exhibit resistance to multiple classes of antimicrobials, leaving certain strains treatable by few antimicrobial therapies and others altogether untreatable (Talyansky et al., 2021). Over the last 40 years, *A. baumannii* has emerged as a difficult-to-treat pathogen due to the global dissemination of multidrug resistant strains (McConnell & Martín-Galiano, 2021), therefor, the rate of mortality has increased (Sarshar et al., 2021). In the last few years *A. baumannii* has become a potential threat to the health of hospitalized patients, especially those in intensive care units (ICUs) (Castro-Jaimes et al., 2022), mainly due to its propensity to acquire multidrug, extensive drug and even pan drug resistance phenotypes at previously unforeseen rates (Harding et al., 2018). It has classified as an ESKAPE pathogen (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumannii, Pseudomonas aeruginosa*, and *Enterobacter* species). Carbapenem-resistant *A. baumannii* is considered the World Health Organization's number one critical priority pathogen for which new therapeutics are urgently required (Morris *et al.*, 2019; Jaloot and Owaid, 2021). Based on the CDC (Centre for Disease Control), two-thirds of all hospital-acquired infections are caused by the six ESKAPE bacteria (Jaloot & Owaid, 2021). In conflict zones, *A. baumannii* is considered as the main cause of concern, and has obtained significant notoriety in the resent desert conflicts in Iraq, earning it the moniker "Iraqibacter''. Consequently, it was noted that the occurrence frequency of multidrug-resistant (MDR) bacteremia was high among members of US Army service following the Operation Iraqi Freedom (Jaloot & Owaid, 2021).

Therefore, this study was aimed to isolate and identify *A. baumannii* in different clinical samples in Erbil (Hawler) hospitals and characterized the isolates using phenotypic and molecular methods.

Chapter Two

2. Literature review

2.1. The genus *Acinetobacter*

2.1.1. Historical perspective of the genus *Acinetobacter*

The history of the genus *Acinetobacter* is confusing and full of uncertainties. Since the early 20th century, and particularly in 1911, a Dutch microbiologist, Beijerinck, designated an organism and gave it the name *Micrococcus calcoaceticus* (Doughari et al., 2011). Similarly 15 genera and species were designated over the next following decades. The most popular designations were *Diplococcus mucosus*, *Micrococcus calcoaceticus*, *Alcaligens hemolysans*, *Mima polymorpha*, *Moraxella lowffii*, *Herella vaginicola*, *Bacterium anitarum*, *Moraxella lowffii var glucidolytica, Neisseria winogradskyi, Achromobacter anitratus,* and *Achromobacter mucosus* (Jung & Park, 2015).The designation *Acinetobacter* was taken from the Greek word ακινετοσ [akinetos], i.e. (non-motile), which was initially proposed by Brisou and Prévot in 1954 in order to differentiate between the motile and non-motile microorganisms belonging to the genus *Achromobacter* (Souli et al., 2008). Baumann in 1968 concluded that all the different species mentioned previously were belonged to a single genus, and because of that the name *Acinetobacter* was proposed (Baumann, 1968). For a long time, this genus belonged to the family Neisseriaceae, but after that it has been listed under the family Moraxellaceae, together with *Moraxella* and *Psychrobacter* (Gordon & Wareham, 2010). The previous findings resulted in the official acknowledgment of the genus *Acinetobacter* by the Subcommittee on the Taxonomy of *Moraxella* and Allied bacteria in 1971. Then the genus *Acinetobacter* was listed in *Bergey's Manual of Systematic Bacteriology* with the description of a separated species called *Acinetobacter calcoaceticus* (Bouvet & Grimont, 1986).

2.1.2. Current taxonomy of the genus *Acinetobacter*

Taxonomy of the genus *Acinetobacter* has a long and complicated history. According to recent taxonomic data, the genus *Acinetobacter* should be classified under the class Gammaproteobacteria within the new family Moraxellaceae (previously the family Neisseriaceae) which consists of the genera *Moraxella*, *Acinetobacter*, *Psychrobacter*, and other related organisms (Visca et al., 2011). Foolowing Jung & Park (2015) the second edition of *Bergey's Manual of Systematic Bacteriology* mentioned that the scientific classification of the genus *Acinetobacter* is as follows (Jung & Park, 2015).

Domain: *Bacteria*

Phylum: *Proteobacteria*

Class: *Gammaproteobacteria*

Order: *Pseudomonadales*

Family: *Moraxellaceae*

Genus: *Acinetobacter*

A magnificent breakthrough was achieved in 1986 by Bouvet and Grimont, who depended on DNA-DNA hybridization studies and DNA sequencing analysis that recognized 12 DNA groups or genospecies, some of which were given formal species names including *A. baumannii, A. calcoaceticus, A. hemolyticus, A. johnsonii, A. junii* and *A. lwoffii* (Bouvet and Grimont, 1986). Currently, there are 33 identified species within the genus *Acinetobacter,* one of them had been named (Nemec et al., 2009). Some *Acinetobacter* species have been classified with official names, these assigned names are: *A. baumannii, A. baylyi, A. beijerinckii, A. bereziniae, A. bouvetii, A. calcoaceticus, A. gerneri, A. grimontii, A. guillouiae, A. gyllenbergii, A. haemolyticus, A. johnsonii, A. junii, A. lwoffii, A. parvus, A. radioresistens, A. schindleri, A. soli, A. tandoii, A. tjernbergiae, A. towneri, A. ursingii* and *A. venetianus* (Visca et al., 2011).

2.1.3. Natural habitat of *Acinetobacter* species

In general, *Acinetobacter* species are isolated from environmental samples like soil and sludge, vegetables, or clinical samples. Numerous members of the genus are regarded as environmental organisms due to their ubiquitous presence in the environment (Peleg et al., 2008). This holds true for the genus *Acinetobacter*, as they can be recovered from virtually all samples obtained from soil or surface water after enrichment (Baumann, 1968). However, not all *Acinetobacter* species have a natural habitat in the environment. *Acinetobacters* are part of the flora of the human skin. In an epidemiological study investigating the colonization of human skin and mucous membranes by *Acinetobacter* species, as many as 43% of non-hospitalized individuals tested positive (Seifert et al., 1997). *A. lwoffii* was isolated the most frequently (58%), followed by A. johnsonii (20%), A. junii (10%), and *A. pittii* (previously genomospecies 3) (6%). In a similar study, 44% of healthy volunteers were found to be carriers, with *A. lwoffii*

(61%), *Acinetobacter* genomic species 15BJ (12%), *A. radioresistens* (8%) and *A. pittii* (5%) being the most common species (Berlau et al., 1999). In hospitalized patients on a regular ward, the carriage rate with these species was even higher, at 75% (Seifert et al., 1997). 25% of healthy individuals carried *Acinetobacter* in their feces, with *A. johnsonii* and *Acinetobacter* genomic species 11 predominating (Dijkshoorn et al., 2005). There were a total of 226 samples examined, in a study of the microbial communities from the extreme environments of six Andean lakes, five distinct *Acinetobacters*, including *A. johnsonii*, were isolated. (Ordoñez et al., 2009). *A. calcoaceticus* species members have been isolated from the soil around plant roots and have been demonstrated to encourage plant development in vitro (Peix et al., 2009). Another soil-isolated microbe, *A. baylyi*, has demonstrated the capacity to acquire plant DNA via horizontal gene transfer or through conjugation. (Pontiroli et al., 2009). It has been demonstrated that an *A. baumannii* isolate from Kuwaiti desert soil polluted with petroleum can break down crude oil (Obuekwe et al., 2009). The most significant nosocomial *Acinetobacter* species*, A. baumannii*, was only infrequently discovered on human skin and in human feces, while *A. nosocomialis* was completely undetectable (Dijkshoorn et al., 2005). Infected animals occasionally contained *A. baumannii* as an etiological agent (Francey et al., 2000). 22% of the body lice samples taken from homeless people contained *A. baumannii* (La Scola & Raoult, 2004). Although it has been suggested that this group may have clinically silent bacteremia, it is not yet apparent what this fact means clinically. In Hong Kong, 51% of local veggies were discovered to be contaminated with *Acinetobacter*, the most of which were *A. pittii* (75%), however one sample grew *A. baumannii* (Houang et al., 2001). In particular, it must be shown that *A. pittii* does not originate from vegetable handling by people. In 22 out of 60 soil samples taken in Hong Kong, *Acinetobacters* were discovered. The most prevalent species were *A. pittii* (27%) and *A. baumannii* (23%); just one sample had *A. calcoaceticus* (Houang et al., 2001). *Acinetobacter* species may readily enter the human food chain via the transfer of infected animals, plants, and water that have been exposed to the bacteria on their surface. Additionally, this pathogenic bacterium may also be disseminated through fomites and silent carriers. *Acinetobacter* was found to be skin-carrying in 44% of healthy persons in a UK research, with *A. lwoffii* accounting for 61% of isolates, *Acinetobacter genospecies* 15BJ for 12.5%, and *A. radioresistens* for 8% (Berlau et al., 1999). A group of healthy individuals had 42.5% of *Acinetobacters*, mostly *A. lwoffii, A. johnsonii*, and to a lesser degree, *A. junii* (Seifert et al., 1997). *Acinetobacter* was

found to infect 53% of medical students and new nurses in the summer compared to 32% of them in the winter, according to Hong Kong research. In the USA, the prevalence of *Acinetobacter* infections showed a seasonal change that was related to higher humidity during the summer (Retailliau et al., 1979).

2.1.4. Species identification

It has proven difficult to identify *Acinetobacter* isolates down to the species level. Broadly, phenotypic schemes are inadequate. Moreover, phenotypic identification using industrial colorimetric techniques has been linked to subpar accuracy (Dijkshoorn et al., 2007). For instance, a 2009 research found that 75% of the isolates utilizing the VITEK 2 GNI identification method had the wrong speciation (Boo, Walsh & Crowley, 2009). On the other hand, the reference standard technique for accurate identification most likely refers to molecular identification of *Acinetobacter* species by DNA-DNA hybridization. Unfortunately, due to its time-consuming, labor-intensive, and limited availability, this approach is inappropriate for use in normal clinical labs (Bergogne-Bérézin & Towner, 1996). As a result, several genotypic techniques have been suggested for the quick and precise identification of *Acinetobacter* species, such as whole-genome fingerprinting, restriction enzyme analysis, or sequence analysis of a specific gene or genetic area (Dijkshoorn et al., 2007). As an alternative, it has been discovered that protein fingerprinting utilizing a MALDI-TOF mass spectrometer is a potential molecular technique for high-throughput, quick identification of *Acinetobacter* species. Previous research demonstrated that a total of 552 well-characterized *Acinetobacter* strains could be divided into discrete clusters representing 15 different species using the MALDI-TOF Mass Spectrometry technique (Karah, 2011). Two PCR-based molecular approaches that are suggested for quickly sorting *A. baumannii* isolates include the identification of the bla_{OXA-51}-like gene intrinsic to the organism and the detection of an internal 208-bp fragment from the 16S-23S rRNA intergenic spacer region(ITS) that is unique to the organism (Turton et al., 2006). Similarly, finding the $bla_{OXA-134}$-like gene intrinsic to *A. lwoffii* may be a potential way to quickly identify isolates that belong to this species (Karah, 2011).

2.2. *Acinetobacter baumannii*

A. baumannii is a gram-negative, non-motile, exclusively aerobic, and coccobacillary bacterium. It may be found in a variety of settings, including water and soil. Moreover, it uses a

wide range of substrates for that growth and may occur at varied temperatures and pH levels (AL-Kadmy et al., 2018).

2.2.1. Natural habitats for *Acinetobacter baumannii*

A diverse collection of organisms known as *Acinetobacter* are generally free-living saprophytes that are widely dispersed across the environment. However different species of the genus are often linked to distinct environments, such as soil, water, sewage, people, foods, and animals (Jung & Park, 2015). According to Munoz-Price and Weinstein (2008), *Acinetobacter spp*. is a common component of the natural flora of the skin, mucous membranes of the throat, and human respiratory secretions. It is a cause of several illnesses (Beggs et al., 2006). The epidermis, oropharynx, and digestive tract are the primary body regions of these bacteria in hospitalized patients (Jung & Park, 2015). *Acinetobacter spp*. were found in healthy subjects' foreheads, noses, ears, throats, tracheas, conjunctiva, hands, vagina, and perineum in a research by Seifert et al. (1997). They also live in moist places such the axillae, the groin, and toe webs (Seifert et al., 1997). Several different species of animals, including birds, fish, and rainbow trout, have been isolated with *Acinetobacter* species (Chahoud, Kanafani and Kanj, 2014; Peleg, Seifert and Paterson, 2008). It has been discovered that *A. baumannii* species are known to contain food. It has been found in a number of foods, including raw fruits, vegetables, milk, and dairy products. *A. baumannii* is commonly isolated from reusable medical devices such ventilator tubing, arterial pressure monitoring equipment, humidifiers, washbasins, plastic urinals, and respirometers since *Acinetobacter spp*. can survive dry environments for extended durations (Chahoud et al., 2014). Additionally, they have been removed from the skin of medical professionals, mattresses, pillows, as well as from all sorts of ventilator equipment and wet environments (Beggs *et al.*, 2006; Chahoud, Kanafani and Kanj, 2014).

2.2.2. *Acinetobacter baumannii* Survival under harshest conditions and resistance to desiccation

Since *A. baumannii* can thrive in nutrient-restricted environments on dry surfaces, this feature enables their persistence and transmission in both natural and medicinal environments. Moreover, contaminated medical tools and equipment could act as reservoirs in lengthy hospital epidemics (Chahoud et al., 2014). Most *A. baumannii* strains can survive on dry surfaces for longer than *E. coli*; some of them may even do so for up to 4 months. Also, *A. baumannii*

persisted on damp and dry surfaces for more than 20 days on glass surfaces when kept at room temperature. The bacterium may transmit illnesses in hospitals because of this property. In reality, the troops' illnesses with *Acinetobacter* were acquired after their admittance to medical institutions rather than from the environment (K. Lee et al., 2011). In the ICU, *Acinetobacter spp*. are more commonly discovered than *Staphylococcus aureus* and *Pseudomonas spp*. on inanimate items and staff members' hands. As they are typically colonized rather than infected, it is difficult to establish the relevance of recovering *Acinetobacter spp*. from clinical samples (Lee *et al.*, 2011; Chahoud, Kanafani and Kanj, 2014).

2.2.3. Characteristics of *Acinetobacter baumannii*

Acinetobacter baumannii is a significant nosocomial pathogen that produces opportunistic infections and is responsible for the bulk of clinical infections caused by *Acinetobacter* species. These infections often affect individuals with impaired immune systems or those in intensive care units (ICU). Old age, early delivery, the extensive use of antibiotics after surgery, indwelling catheters, wounds, and the duration of hospital and/or ICU stay are other risk factors that enhance the incidence of *A. baumannii* infection (García-Garmendia et al., 2001). *A. baumannii* is a significant contributor to hospital-acquired pneumonia, especially in those who need mechanical ventilation (Peleg et al., 2008). Moreover, this bacteria is often linked to skin infections, especially burns or surgical wounds, and severe wound infections may result in bacteremia (Antunes et al., 2014). In certain cases, *A. baumannii* causes urinary tract infections and is also a possible cause of secondary meningitis. There have been a few reports of community-acquired *A. baumannii* infections, and heavy drinking is one risk factor (Anstey et al., 2002). *It* is not often isolated from non-clinical environmental sources, unlike other species of *Acinetobacter*, and its natural reservoir is unknown (Towner, 2009; Visca, Seifert and Towner, 2011). *A. baumannii* may be found on hospital furniture, patient and staff skin, and hospital surfaces during an epidemic. As a result of their high degree of resistance to desiccation and disinfection, once these bacteria are established in the hospital environment, they are particularly challenging to eliminate (Towner, 2009). Although it was this second characteristic that prompted specialized studies of *A. baumannii* in the 1990s, particularly those that were carbapenem resistant, it is likely that *A. baumannii's* success as a nosocomial pathogen is due to both its ability to persist in the hospital environment and its high level of antibiotic resistance (Towner, 2009). Current isolates frequently only remain susceptible to colistin (Antunes et al.,

2014) and ominously, isolates resistant to all antibiotics have been detected (Göttig et al., 2014). According to a World Health Organization assessment in 2017, the danger posed by *A. baumannii* that is carbapenem-resistant is severe and new therapies must be developed immediately. Antibiotic resistance in *A. baumannii* was first classified and described using standardized nomenclature in 2011, which allowed for reliable global comparison of surveillance data (Magiorakos et al., 2012). This categorization system was based on the inability to cure *Acinetobacter* infections with 9 classes of effective antibiotics (Magiorakos et al., 2012). Multiple antibiotic resistance (MAR) was defined as the ability of the bacteria to resist one or more antibiotics in at least three categories, extensive antibiotic resistance (XAR) as the ability of the bacteria to resist an antibiotic in seven out of nine categories, and pan antibiotic resistance (PAR) as the ability of the bacteria to resist all antibiotics (PAR) (Magiorakos et al., 2012).

2.2.4. Virulence of *Acinetobacter baumannii*

Acinetobacter baumannii was formerly thought to be a low-virulent bacterium, but the prevalence of infections like community-acquired *Acinetobacter* pneumonia shows that it is highly pathogenic and causes invasive illness. Lack of knowledge of the host's molecular and cellular pathways of pathogenesis makes treating *A. baumannii* infections difficult. The virulence components of *A. baumannii* that contribute to the formation of biofilms, the destruction of host cells, and infection still need a great deal of research (Gaddy & Actis, 2009). *A. baumannii* must possess a number of virulence factors in order to colonize and infect the host. About these virulence factors in *A. baumannii*, however, not much is known. Several virulence factors, including phospholipases D and C, biofilm development, outer membrane protein A (OmpA), penicillin-binding protein 7/8 (PBP-7/8), and ferric iron chelators (siderophores) have been the subject of recent investigations (Roca Subirà et al., 2012). The surface protein OmpA is involved in the adherence to and invasion of epithelial cells and induces apoptosis in the early stages of *A. baumannii* infection (McConnell et al., 2011), whereas the hydrolytic enzymes Phospholipase C and Phospholipase D (PLD and PLC) help to increase cytotoxicity on epithelial cells, invade epithelial cells, and allow the organism to thrive in human blood. *A. baumannii* develops a biofilm, a highly organized microbial community, during colonization, which promotes adherence to host cells, survival on various surfaces, and resistance to antimicrobial treatments (Gaddy & Actis, 2009). Penicillin-binding protein 7/8 (PBP-7/8) contribute in the growth and

survival of *A. baumannii* in human ascites, and showed indirect participation in human serum resistance (Cayô et al., 2011).

2.3. Porins

The outer membrane serves as Gram-negative bacteria's initial line of defense against hazardous substances. Large, charged molecules cannot pass through this barrier. Porins, which are water-filled open channels that span the outer membrane and permit the passive passage of hydrophilic molecules, play a significant role in controlling the permeability of the outer membrane (Galdiero et al., 2012). Porins and efflux pumps are examples of outer membrane proteins (OMPs), which play a crucial role in the pathogenicity and antibiotic resistance of the organism. Antibiotic resistance to a variety of hydrophilic antibiotics, such as -lactams and carbapenems, has been linked to altered porin expression. Three porins have been found in *A. baumannii*: CarO (Catel-Ferreira et al., 2011), OprD (Catel-Ferreira et al., 2012), and 33kDa porin (Clark, 1996). These porins showed association to carbapenems resistance in *A. baumannii* (Del Mar Tomas *et al.*, 2005).

2.4. Quorum Sensing

Bacterial QS is a method of cell-to-cell communication based on particular signaling molecules known as "auto-inducers" that enables bacteria to detect population densities. The expression of virulence factors, motility, conjugation, biofilm formation, and interactions with eukaryotic host cells are all crucially influenced by QS systems (Zarrilli, 2016; Eze, Chenia and El Zowalaty, 2018; Colquhoun and Rather, 2020). *A. baumannii* now only has one QS system, which is made up of the genes abaI and abaR that were horizontally transferred from *Halothiobacillus neapolitanus*. The autoinducer synthase, AbaI, and its corresponding receptor, AbaR, are members of the LuxI/LuxR family, which is often seen in other Gram-negative bacteria. When N-(3-hydroxydodecanoyl)-1-homoserine lactone (also known as Acyl Homo-serine Lactones, AHLs) binds to AbaR, the complex identifies lux-box sequences on QS target promoters and controls the expression of those promoters (Saipriya et al., 2020). In fact, QS molecules influence the bfmS and bfmR genes of *A. baumannii* by upregulating their expression, which promotes the production of potent biofilms on abiotic surfaces. Moreover, insufficient iron concentrations upregulate QS signaling molecules, increasing the virulence and persistence of *A. baumannii* (Eze, Chenia & El Zowalaty, 2018; Modarresi *et al.*, 2015).

2.5. Biofilm formation

To protecting the bacteria and enhancing its capacity to acquire foreign genetic material through horizantal gene transfer, biofilms (communities of organisms attached to a surface) enable the organism to persist in hospitals on various medical surfaces (cardiac valves, artificial joints, catheters, endotracheal tubes, and ventilators). They also enable the organism to evade antibiotics. *A. baumannii* forms biofilms more quickly than other species do. Recent studies have shown how the ability of *A. baumannii* to form biofilms influences resistance phenotypes, resistance development, and spread within biofilms through conjugation or transformation, so making biofilms a hotspot for genetic exchange. *A. baumannii* biofilm formation is regulated by a number of genes, and the organism exhibits a positive correlation between biofilm development and ‘antimicrobial resistance’ (AMR) (Roy et al., 2022).

2.6. Mechanisms of antimicrobial resistance in *Acinetobacter baumannii*

Most of the existing antibiotics are no longer effective against *A. baumannii. A. baumannii's* drug resistance may be brought on by either intrinsic processes seen in Gram-negative organisms or through horizontal gene transfer's acquisition of new genetic information (H et al., 2003). Antibiotic resistance mechanisms for *A. baumannii* can be classified into three categories (Piddock, 2006). Production of enzymes that hydrolyze and give resistance to penicillins, cephalosporins, and carbapenems (β-lactamases, cephalosporinases, and carbapenemases) is the first group (Drawz et al., 2010). Inactivation of the medication by β-lactamase enzymes, which may be either chromosomally or plasmid-encoded, is the most typical method of β-lactam resistance in *A. baumannii* (Roca et al., 2012). The second category involves modification of membrane permeability or increased efflux (Limansky *et al.,* 2002); however, little is known about the permeability of the outer membrane in *A. baumannii* or its outer membrane porins (Vila et al., 2007). This sort of mechanism in *A. baumannii* confers resistance to quinolone drugs through mutations in the bacterial targets gyrA and parC topoisomerase enzymes, falling under the third group of resistance mechanisms. These changes affect the targets of antibiotics or the functionality of bacterial cells (Maragakis & Perl, 2008). Overall, *A. baumannii* is capable of expressing all of the resistance mechanisms mentioned above, including enzymatic inactivation of the antibiotic, mutations in the target protein's structural or regulatory genes, decreased permeability of the outer membrane, and efflux transport of the agent out of the

cell (Roca et al., 2011). However recent research has shown that the energy-mediated efflux of antibiotics is present and plays a significant part in the intrinsic resistance of Gram-negative organisms (Lin et al., 2009). *A. baumannii* has efflux pumps, which are responsible for this organism's multidrug resistance phenotype and are capable of actively removing a wide variety of antimicrobial drugs from the bacterial cell (Damier-Piolle et al., 2008).

2.7. Resistance-Nodulation Cell-Division (RND) efflux pumps

RND family multidrug efflux pumps have been discovered as the most prevalent and significant efflux mechanism among MDR Gram-negative bacteria (Morita et al., 2012). An outer membrane protein (OMP), an inner membrane RND transporter, and a membrane fusion protein (MFP) that connects the OMP and RND components are the components of RND pumps. In addition to the efflux of antibiotics, RND pumps have also been linked to a wide range of structurally unrelated molecules and ions, including biocides, heavy metals, dyes, organic solvents, detergents, metabolic inhibitors, bile salts, and homoserine lactones, which are used in quorum sensing. RND pumps are powered by a substrate/H+ antiport mechanism. To date, three RND-efflux pumps have been described in *A. baumannii AdeABC* (Xu et al., 2019), *AdeIJK* (Damier-Piolle et al., 2008) and *AdeFGH* (Coyne et al., 2010). A number of antibiotic families, including beta-lactams, aminoglycosides, fluoroquinolones, tigecycline, trimethoprim, and sulfamethoxazole have proven that, these three efflux pumps are capable of conferring resistance (Xu et al., 2019).

2.8. Antimicrobial resistance in *Acinetobacter baumannii*

2.8.1. Resistance to β-lactam:

Examples of beta-lactam antibiotics include penicillin, cephalosporins, carbapenems, monobactams, and beta-lactamase inhibitors. The d-Ala-d-Ala moiety of peptidoglycans is similar to the structure of beta-lactams. They therefore attach to penicillin-binding proteins in bacterial cell walls and stop the transpeptidation process, which is the last step in the production of peptidoglycans (Kyriakidis et al., 2021). There are a number of fundamental processes that underlie bacteria's resistance to beta-lactams. Production of the β-lactamase enzyme is the most prevalent form of resistance in Gram-negative bacteria (H. I. Hussain et al., 2021). *A. baumannii* is nowadays intrinsically resistant to penicillins and cephalosporins (Kyriakidis et al., 2021).

There are a number of fundamental mechanisms for β-lactams resistance in bacteria. The most prevalent form of resistance in Gram-negative bacteria is the development of the β-lactamase enzyme. Several Gram-negative bacteria have naturally occurred, chromosomally mediated β-lactamases that have been characterized as aiding the microbes in either competing with β-lactam generating bacteria or removing β-lactam-like compounds that may be employed as regulators of cell wall formation. It has been claimed that bacteria produce at least 400 distinct kinds of β-lactamase (Carcione et al., 2021). β-lactamases are enzymes that catalyze the hydrolysis of β-lactam antibiotics and can be divided into four classes based on sequence motifs and differences in hydrolytic mechanism according to ambler classification (table 2.1) (Kyriakidis et al., 2021).

Table 2. 1. β-lactamase classification according to ambler classification (Kyriakidis et al., 2021).

Ambler class	Active site	Type	Enzyme type	Resistance to antibiotics
A	Serine	Serine β-lactamase	Narrow spectrum β-lactamase and extend β-lactamase (TEM, SHV, CTX-M …etc.)	Hydrolyze penicillin, cephalosporins, carbapenems and β-lactams
B	Metal (zinc or other heavy metal)	Metallo β-lactamase	VIM, IMP, NDM...etc.	Carbapenems
C	Serine	Serine β-lactamase	AmpC	Cephamycin and cephalosporins
D	Serine	Serine β-lactamase	OXA	Oxacillin, oxyimino β-lactams and carbapenems

2.8.2. Resistance to Aminoglycosides

Acinetobacter baumannii resistance to aminoglycosides (AG) can be caused by three different mechanisms: aminoglycoside-modifying enzymes (AMEs), which reduce AG binding capacity, 16S rRNA methyltransferases, which alter the target site, and limited AG uptake due to decreased permeability or excessive efflux pump activity (Kyriakidis et al., 2021). Three different functional groups of modifier enzymes are known including, aminoglycoside acetyltransferases (AAC), such as AAC (60)-Ih (which also confers resistance to gentamicin and amikacin), aminoglycoside phosphotransferases (APH), such as APH (30)-IA (which confers resistance to gentamicin), and aminoglycoside adenylyltransferase (ANT), such as ANT (200)-

IA (Vázquez-López et al., 2020). AG resistance genes can be transferred by means of mobilizable or conjugative plasmids, natural transformation, or transduction (Garneau-Tsodikova & J. Labby, 2016). While AMEs continue to be the major method used by *A. baumannii* to avoid removal by AGs, efflux is also a new issue with AG usage. Effective efflux of AGs is caused by a variety of gene products, including pumps, permeases, periplasmic adaptors, and two component systems (TCSs) (De Silva & Kumar, 2019). One thing to notice is that whereas gentamicin and netilmicin are efficiently removed by the AdeABC and AbeM pumps, efflux is much weaker in the case of more hydrophilic AGs like amikacin and kanamycin (Xu et al., 2019). Porin expression and changes in membrane lipids are very minor mechanisms of AG resistance that are currently being studied (Garneau-Tsodikova & Labby, 2016).

2.8.3. Resistance to tetracycline:

Currently under investigation are two relatively small mechanisms of AG resistance: porin expression and modifications to membrane lipids (Chukwudi, 2016). Three major pathways are thought to be responsible for antibiotic tetracycline resistance: Tetracycline inactivation via enzymes, ATP-dependent efflux and ribosomal protective proteins (RPPs) (Warburton et al., 2016). TetA and TetB, which are both particular transposon-mediated efflux pumps, have been reported to be two separate forms of specialized *A. baumannii* tetracycline resistance based on efflux pumps or a RPP. TetB regulates the efflux of tetracycline as well as minocycline, whilst TetA exclusively regulates the efflux of tetracycline. The second mechanism is the RPP, that shields the ribosome from the effects of tetracycline. This protein, which is produced by the tet(M) gene, protects the ribosome against the effects of tetracycline, doxycycline, and minocycline (Falagas et al., 2015a). The TetM determinant has also been seen and confers resistance through ribosomal protection. Resistance to tigecycline (Perez et al., 2007), a modified tetracycline known as a glycycline, is often associated with overexpression of AdeABC and AdeIJK efflux pumps (Ribera et al., 2003). As most clinical isolates of *A. baumannii* exhibit efflux pumps of the RND type, tigecycline is susceptible to effusion caused by these pumps (Vázquez-López et al., 2020). Tetracyclines may be efficiently eliminated by RND pumps, namely AdeABC, but they also significantly raise the minimum inhibitory concentrations (MICs) for tigecycline, minocycline, and tetracycline (Xu et al., 2019). Pump RND AdeIJK may work in concert with other overexpressed efflux pumps (such as AdeABC and

AcrAB-TolC) to cause tigecycline resistance, despite the fact that it seems to have a small role in tetracycline resistance against *A. baumannii* (Yuhan et al., 2016).

2.8.4. Resistance to quinolones and fluoroquinolones:

Fluoroquinolones, which make up the majority of quinolone antibiotics, have a wide range and are effective against both Gram-negative and Gram-positive infections. Quinolone antibiotics prevent bacterial DNA from loosening and being copied, which is how they stop DNA replication. Quinolones work by preventing the type II topoisomerases, DNA gyrase, and topoisomerase IV from acting as ligases, which ordinarily cause supercoiling in conjunction with DNA nucleases. When ligase function is disrupted, bacteria continue to have double-stranded DNA breaks, which causes them to die (Aldred et al., 2014). Three different mechanisms contribute to quinolone resistance: first target mutations in gyrase and topoisomerase IV, which reduce the strength of their respective quinolone-enzyme interactions; second plasmid-borne resistance mediated by Qnr proteins, the AMEs AAC(60)-Ib-cr and AAC(60)-Ib-cr5; and the last one chromosome-derived resistance caused by either low expression (Kyriakidis et al., 2021). Fluoroquinolone resistance in *A. baumannii* is caused by mutations in DNA gyrase and DNA topoisomerase IV's quinolone resistance-determining regions (QRDRs), which prevent the fluoroquinolones from binding to their intended target proteins. In strains with RDRQ mutations, overexpression of efflux active pumps may exacerbate resistance and itself produce mild resistance (Kyriakidis et al., 2021). Mutations in the bacterial gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parA* and *parC*) genes may result in quinolone and fluoroquinolone resistance by decreasing the binding affinity of these antibiotics to the enzyme-DNA complex (Poirel et al., 2011). Ser-81-Leu substitutions in GyrA and Ser-84-Leu substitutions in ParC are the most frequently reported mutations that have been experimentally shown to give resistance to fluoroquinolone antibiotics like ciprofloxacin. Fluoroquinolone resistance in *A. baumannii* was documented in 50% to 73% of cases, but it has significantly increased in poor nations during the last several years, reaching 75% to 97.7% of cases (Zaki, Abou ElKheir and Mofreh, 2018; Vázquez-López *et al.*, 2020).

2.8.5. Resistance to Macrolides:

The use of macrolide antibiotics in *A. baumannii* infections is limited. Azithromycin seems to prevent the synthesis of mucin, but no other macrolide does, indicating that it is

effective in treating ventilator-associated pneumonia. For treating pediatric and adult patients in ICUs with nosocomial pneumonia caused by *A. baumannii*, azithromycin is used with additional antibiotics (Yamada et al., 2014). The ABC-F type ribosomal protection protein Msr(E) or msr(E), two macrolide 2' phosphotransferases encoded by mph(A) and mph(B), and three 23S rRNA (adenine(2058)-N(6))-methyltransferases are responsible for *A. baumannii's* resistance to macrolides, according to MicroBIGG-E database (E) (Kyriakidis et al., 2021). The first two classes lead to resistance by modification of the target site, while the third-class results in macrolide inactivation. Mph(A) and Mph(E) in particular seem to provide resistance to erythromycin, clarithromycin, azithromycin, and oleandomycin, but only when certain regulatory proteins are present (Cheng et al., 2016).

2.8.6. Resistance to colistin:

Lipid A (LPS) is modified in the fundamental mechanism of polymyxin resistance, reducing the outer membrane's net negative charge (Moffatt et al., 2019). Increases in colistin MICs have been linked to point mutations and frameshifts in the *pmrA* and *pmrB* genes, two parts of a system that controls the modification of LPS (Qureshi et al., 2015). There is evidence that the full loss of LPS caused by mutations in lipid A production genes may potentially contribute to colistin resistance (*lpxA, lpxC and lpxD*) (Vila-Farrés et al., 2015).

2.8.7. Resistance to sulfonamides and trimethoprim:

Sulphonamide resistance is frequently detected in *A. baumannii* (Poirel, et al., 2011). Class 1 integrons which harbour the sul1 resistance gene in the 3`-conserved segment (3`-CS) are frequently detected (Sung *et al.*, 2014; Holt *et al.*, 2015). Sulphonamide resistance in *Acinetobacter* can also be conferred by the sul2 gene (Nigro & Hall, 2012), in a segment formed from integrase of genomic island sul2, which is often next to a complete or partial Common region (CR2) (GIsul2) (Nigro & Hall, 2011). *Dfr* genes found in gene cassettes in integrons or the overexpression of intrinsic efflux pumps may be linked to resistance to trimethoprim. (Coyne et al., 2011).

2.9. Clinical manifestations of *Acinetobacter baumannii* infections

The multi-resistant bacterium *A. baumannii* is quickly establishing itself as the model for germs that are resistant to antibiotics. The severity of the clinical condition and medication resistance are the two main issues that doctors in ICUs must deal with (Peleg, Seifert and

Paterson, 2008; Lee *et al.*, 2010). *Acinetobacters,* which colonize the skin and mucous membranes of patients and are to blame for the development of nosocomial infections, are often discovered in hospitals. Resistance to potent antimicrobial agents, desiccation and disinfectants are the three main causes of this bacterium's power (Bergogne-Bérézin et al., 2008). It has been provided evidence that MDR *A. baumannii* infections are associated with a mortality rate in excess of 24% (Weinberg et al., 2020) . Other studies report a crude mortality rate of 26%–52% (Asif et al., 2018). *A. baumannii* has been identified in several parts of the globe, and in recent years, it has been linked to high rates of illness and death. (Kyriakidis et al., 2021). Its total mortality rate may reach 56.2% (Mohd Sazlly Lim et al. 2019). In the eastern Arab nations, there has been an increase in the occurrence of resistant *A. baumannii* isolates (Iraq, Jordan, Lebanon, Palestinian, and Syria) (Moghnieh *et al.*, 2018; Hussain *et al.*, 2022). *A. baumannii* infections were observed among badly wounded returning Canadian and American troops during the wars in Iraq and Afghanistan (Tien et al., 2007). It was speculated that this had significantly contributed to the rise in *A. baumannii* infections among hospitalized civilians in North America (Hujer et al., 2006a).

2.9.1. Urinary tract infections

Just 1.6% of UTIs acquired in ICUs are caused by *A. baumannii*, which is often an infection or colonization related to catheters. Nonetheless, the prevalence of UTI brought on by *A. baumannii* has gradually increased (Gaynes et al., 2005). Opportunistic bacteria frequently colonize the location when endotracheal tubes, intravascular, ventricular, or urinary catheters are used (Joly-Guillou, 2008). It is not unusual for this bacterium to cause uncomplicated UTI in healthy outpatients (Peleg et al., 2008).

2.9.2. Meningitis

Acinetobacter baumannii meningitis is becoming an increasingly important entity with mortality rates as high as 64% been reported in patients with meningitis due to *A. baumannii* (García-Garmendia et al., 2001). Sporadic cases have been reported following neurosurgical procedures (Chen et al., 2005). Following the delivery of intrathecal methotrexate to a group of children with leukemia, an epidemic of *A. baumannii* meningitis was reported due to improperly sanitized needles, which resulted in the deaths of three children. Meningitis risk factors include the use of antibiotics, a ventriculostomy, a CSF fistula, an indwelling ventricular catheter for

longer than five days, and a continuous link between the ventricles and the outside environment. Turkey has had a high frequency of meningitis caused by multi-resistant *Acinetobacter* in patients undergoing neurosurgery (Metan et al., 2007). The surveillance of local pathogens in neurosurgical wards should guide the selection of proper therapy with an effective infection control program (Joly-Guillou, 2008).

2.9.3. Suppurative infections

Acinetobacter infections of the skin and soft tissues have recently become exceedingly troublesome. This may be because strains of *Acinetobacter* species are the only category of Gram-negative bacteria that may be found naturally on the human skin (Seifert et al., 1997). According to Gaynes and Edwards (2005), *Acinetobacter* caused 2.1% of skin/soft tissue infections that occurred in ICUs (Gaynes et al., 2005). It is a very difficult pathogen to eradicate from a burns unit (Trottier et al., 2007). *A. baumannii* was found to frequently be isolated from the wounds of war fatalities from Iraq or Afghanistan (Peleg et al., 2008). While it was frequently isolated from patients with open tibial fractures, its poor pathogenicity at this location allowed it to be totally eliminated (Johnson et al., 2007).

2.9.4. Bloodstream infections

It was shown that 1.3% of all nosocomial bloodstream infections in the United States were caused by *A. baumannii*, the tenth most frequent aetiologic agent (Peleg et al., 2008). Bloodstream infections obtained in ICUs were more frequently caused by *A. baumannii* than infections acquired in non-ICU wards (1.6% versus 0.9% of bloodstream infections in those settings, respectively). *A. baumannii* bloodstream infection caused an overall crude death rate of 34.0% to 43.4% in the ICU and 16.3% outside of the ICU. Only *P. aeruginosa* and *Candida spp*. infections caused bloodstream infections with a higher crude death rate in the ICU than *A. baumannii*. At a mean of 26 days following hospital admission, *A. baumannii* infections were the most recent bloodstream infections to develop during hospitalization (Peleg et al., 2008). It is therefore not certain if the high crude mortality rate represents its occurrence in patients with ongoing underlying critical illness or whether the organism does have significant attributable mortality. It is notable that 102 patients had bloodstream infections at sites treating U.S. military members injured in Iraq or Afghanistan from 1 January 2002 and 31 August 2004 (Peleg et al., 2008). *A. baumannii* is believed to be resistant to a variety of antibiotics, and most crucially, *A.*

baumannii bacteremia has a poorer prognosis than bacteremia caused by non-*A. baumannii* isolates (Nogbou et al., 2010).

2.9.5. Wound and burn infections

A. baumannii is also an important cause of burn infections in hospital settings. The widespread of this organism in burn units often had been related to the contamination of the hospital environment and the spreading of resistant strains (Joly-Guillou, 2008). It has been mentioned previously that the outbreak of *A. baumannii* was known at first in Iraq by the United States military operations (Perez et al., 2010), and since then it is developing such a terrible reputation among injured soldiers during the Iraqi war (after 2003) and it had the nickname '*Iraqibacter*' due to the spreading of clones from Iraq to American military hospitals in Germany and US (Petersen *et al.*, 2007; Peleg, Seifert and Paterson, 2008). Wound infections caused by multidrug-resistant *A. baumannii* represent a major problem with regard to morbidity and mortality in both civilians and wounded military service members (Dallo and Weitao, 2010; Michalopoulos and Falagas, 2010). Burn infection may delay the wound healing and cause a failure in skin transplants. Moreover, the colonization of wound site can lead to the infection of the underlying tissue which finally causes the subsequent systemic spread of the bacteria (Trottier et al., 2007). Soft tissue infections due to *A. baumannii* have emerged as a significant dilemma in military personnel suffering from war-related trauma in Iraq and Afghanistan (Sebeny, Riddle and Petersen, 2008; Falagas *et al.*, 2015b). Wound infections occur at higher rates among military service members, possibly because of problems related to pre-treatment time, type of traumatic injury and the fact that the patients will pass through multiple medical facilities before arriving to the main treatment facility (Keen et al., 2010). *A. baumannii* has been also associated with pressure ulcers (bedsores) and wounds in people with diabetes (Boyanova & Mitov, 2014).

2.9.6. Pneumonia

Hospital acquired-pneumonia is the most common clinical manifestations of *A. baumannii*. The majority of *A. baumannii* infections are from the respiratory tracts of hospitalized patients (Gaynes et al., 2005). This might be associated with the colonization of airways by this bacterium via environmental exposure, which soon develops into pneumonia especially in patients who received mechanical ventilation in ICUs (Peleg, Seifert and Paterson, 2008; Doughari *et al.*, 2011). Ventilator-associated pneumonia (VAP) caused by *A. baumannii*

has a mortality rate ranging from 40% to 70% (Garnacho et al., 2003). Community-acquired *A. baumannii* pneumonia is a minimal frequent infection with a 40% to 60% mortality rate (Leung et al., 2006). This disease most typically occurs during the rainy season and associated with underlying host factors such as alcohol abuse or chronic obstructive pulmonary disease (McConnell et al., 2013).

2.10. COVID-19 and *Acinetobacter baumannii* Coinfections:

Late in 2019, COVID-19 began to spread globally, causing catastrophic public health crises and much higher rates of morbidity and death, particularly in senior populations and people with serious medical comorbidities. The symptoms of COVID-19 illness are variable and may present differently in each patient. In severe circumstances, patients may experience multi-organ failure, acute respiratory distress syndrome, and pneumonia (Rangel et al., 2021). An urgent public health problem is the carbapenem-resistant *A. baumannii* (CRAb), an opportunistic bacteria predominantly linked to infections acquired in hospitals (Rangel et al., 2021). CRAb outbreaks in acute care hospitals are difficult to contain because it rapidly contaminates the hospital setting and healthcare workers' hands, may persist for extended periods on dry surfaces, and can spread through asymptomatic colonization (Nutman et al., 2016). Many immunocompromised people were admitted to hospitals because of the COVID-19 pandemic, and some reports claimed that some of these patients had secondary illnesses and coinfections (Rasmussen *et al.*, 2020; Ritchie and Singanayagam, 2020; Kyriakidis *et al.*, 2021). There is a significant gap in the information about the incidence, prevalence, and features of bacterial infection in these individuals (Huttner *et al.*, 2020; Cox *et al.*, 2020). Although the precise form and origin of these diseases have not yet been completely analyzed, evidence suggests that multidrug-resistant bacteria are among the germs that cause these infections. In other investigations, the frequency of coinfections among COVID-19 patients varied. Yet, it can be as high as 50% for those who do not survive (Lai et al., 2020). During the COVID-19 pandemic, many instances of coinfection with *A. baumannii* related to SARS-CoV-2 infections have been documented in the literature, including Wuhan (China), France, Spain, Iran, Egypt, New York (USA), Italy, and Brazil. *A. baumannii*-related secondary infections, primarily lower respiratory tract infections, were reported to affect up to 1% of COVID-19 patients admitted in an Italian hospital (Ripa et al., 2021). A descriptive study reported the exact incidence (1%) among hospitalized patients from Wuhan, China (Chen et al., 2020). One out of every 69 hospitalized

patients (1.4%), according to a concurrent Wuhan study, had COVID-19 and *A. baumannii* coinfection (Wang et al., 2020). In addition, a recent French study found that patients with severe SARS-CoV-2 pneumonia in ICU had a 28% risk of bacterial coinfection with *A. baumannii* at 1 out of 92 (1.1%) (Contou et al., 2020). The prevalence and features of respiratory coinfections in COVID-19 patients in the ICU were examined in a research by Siyuan et al. (2021); they found that *A. baumannii* and *S. aureus* were more commonly found during late ICU admission (Yang et al., 2021).

Critically sick COVID-19 patients frequently have respiratory tract distortion or pulmonary dysbiosis in the first few days after contracting SARS-CoV-2, which can eventually evolve into a secondary bacterial or fungus infection a few weeks later (Shi *et al.*, 2020; Rawson *et al.*, 2020). At Cambridge university, UK, a retrospective cohort study in a secondary care environment found that 9 out of 14 COVID-19 patients in the ICUs developed secondary ventilator-associated pneumonia (VAP) that was verified (Hughes et al., 2020). *A. baumannii* was determined to be the causative culprit in a COVID-19 patient with VAP as reported by Lescure et al. (2020). In a retrospective observational analysis of all COVID-19 patients admitted to a university hospital in Spain, *A. baumannii*, which is antibiotic resistant, emerged as the primary cause of respiratory infections and bacteremia, and 16% of patients had fungal or bacterial coinfections or superinfections (Nebreda-Mayoral et al., 2022). Also, according to Chen et al. (2020), COVID-19 patients who had bacterial and fungal coinfections have a greater risk of getting septic shock. One of these patients had an infection with the fungus *A. baumannii* that was extremely resistant to medicines when they first appeared (Ripa et al., 2021). In a cohort study, the authors examined data from 212 critically ill COVID-19 patients who were admitted to a public tertiary hospital that was solely responsible for caring for COVID-19 patients during the pandemic and examined the relationship between bacterial and fungal coinfections and patient mortality. The third-highest death rate of COVID-19 patients with these coinfections was caused by *Acinetobacter spp*., which was the second-most isolated of the patients with positive bacterial cultures (Silva et al., 2021).

Despite significant levels of resistance to all antimicrobials tested, with the exception of colistin, which showed a resistance rate of 52%, possibly an ICU Iranian researcher observed coinfection with MDR *A. baumannii* in 17 out of 19 COVID-19 patients, and none of the patients

survived (Sharifipour et al., 2020). 102 (6.8%) of the 1495 COVID-19 patients hospitalized in Wuhan developed secondary bacterial infections, the majority of which were caused by *A. baumannii* (35.8%), which has a high incidence of resistance (91.2%), and nearly half of them (49.0%, 50/102) passed away while they were receiving treatment (Li et al., 2020). The fast growth of the ICU in response to the management of SARS-CoV-2 has the potential to raise the risk of nosocomial infections in the hospital setting. Bacterial coinfections in COVID-19 patients are therefore a major cause for worry since they greatly raise the morbidity and mortality of these patients. Early detection of bacterial infections would therefore aid in identifying high-risk patients and choosing the most effective therapies to lower mortality (Rangel et al., 2021).

Chapter Three

3. Material and methods

The laboratory instruments and equipment, culture media and chemical materials, as well as kits and materials of molecular characterization which used in this study are illustrated in tables 3.1, 3.2, 3.3 and 3.4.

3.1. Materials

3.1.1. Apparatus and Equipment

The apparatuses and equipments used in the study are shown in table 3.1.

Table 3. 1. The apparatuses and equipments.

No.	Name	Manufacture Company	Country
1.	Incubator	LabTec	Korea
2.	Autoclave	LabTec	Korea
3.	Centrifuge	Truip International Corp	Korea
4.	Auto Vortex	Stuart Scientific	UK
5.	Eppendorf centrifuge	Eppendorf	Germany
6.	Sensitive Balance	LabTec	Korea
7.	Micropipette 0.5-10 ,1-20 ,5-50 10-200 ,100-1000 µl	Slamed and Eppendorf	Germany
8.	Electrophoresis Power supply& Horizontal Gel Tank	Aplex	France
9.	Thermocycler	CrealCon Technologies	The Netherlands
10.	UV Viewing Cabinet	Bijing Linyi	China
11.	Camera	Sony	Japan
12.	Microwave	LG	Korea
13.	Hotplate Stirrer	LabTec	Korea
14.	Refrigerator	Hitachi	Japan
15.	Water Distillater	LabTec	Korea
16.	pH meter	W T W	Germany
17.	Oven	LabTec	Korea
18.	Micropipette Tips	Accumaxtips	Germany
19.	Compound Light Microscope	Olympus	Japan
20.	Ultracentrifuge	Sigma	USA
21.	Chest Freezer	Shownic	Malaysia
22.	Test tube	Indiamart	India
23.	Disposable Petri dishes	Indiamart	India
24.	Mini vortex	Lab genius	UK
25.	NanoDrop 2000/2000c spectrophotometer	Thermo Fisher Scientific	USA
26.	PCR System	PCRmax	UK
27.	Vitek system, Vitek glass, test tubes, Vitek Gram negative ID Card	BioMerieux	France
29.	Sterilized cotton swabs	Indiamart	india

30.	Urine container	Indiamart	india
31.	Plane tubes	Indiamart	india

3.1.2. Chemicals and solutions

The Chemicals and solutions used in the study are shown in table 3-2.

Table 3. 2. The chemicals and solutions

No.	Name	Manufacture Company	Country
1.	Agarose powder	Fermentas	Germany
2.	Loading dye 6X	Fermentas	Germany
3.	Ethanol (99.9%)	sharlau	spain
4.	Ethidium Bromide	Sigma	Germany
5.	Glycerol	Fisong	England
6.	Normal saline	ADWIK	Egypt
7.	Oil immersion	BDH	England
8.	Tris-borate-EDTA buffer TBE Buffer	Fermentas	Germany
9.	Urea crystal	Fermentas	Germany
10.	Glucose powder	Fermentas	Germany
11.	Ladder DNA	SMOBIO	Germany
12.	Safe dye	SMOBIO	Germany
13.	Master mix	Ampliqon	Denmark
14.	Free nuclease water	Thermo Fisher Scientific	USA

3.1.3. Culture Media and kits:

The culture media and kits used in the study are shown in table 3.3 and 3.4.

Table 3. 3. Culture Media

No.	Culture Media	Company	
1.	Brain heart infusion broth	HiMedia	India
2.	Blood agar	HiMedia	India
3.	MacConkey agar	HiMedia	India
4.	Nutrient broth	HiMedia	India
5.	Nutrient agar	HiMedia	India
6.	Kligler iron agar	HiMedia	India
7.	Simmon citrate agar	HiMedia	India
8.	Urea agar	LabM	UK
9.	Kovacs Indol Reagent	HiMedia	India
10.	Peptone Water	LabM	UK
11.	Blood agar	HiMedia	India

Table 3. 4. Kits:

No.	Kits	Company

1.	Genomic DNA extraction kit	POUYA GENE AZMA CO. (Iran)
2.	Plasmid DNA extraction kit	Gene aid (Taiwan)
3.	Gram stain kit	Crescent diagnosis company, KSA

3.2. Method:

3.2.1. Culture Media

Growth media was prepared according to the instructions provided by the manufacturer, and autoclaved at 121°C for 15 minutes under 15 pound per square inch of pressure.

3.2.1.1. Brain heart infusion broth

The medium was prepared by dissolving 37 g of the powder in 1litter of distilled water, autoclaved. Then it was distributed into 30 mL screw cap bottles.

3.2.1.2. Blood agar

The blood agar was prepared by dissolving 40 g of the blood agar base in 1.0 L of distilled water, autoclave, human blood (7%) was added after cooling to 50˚C, according to manufacturer.

3.2.1.3. MacConkey′s agar

The MacConkey’s agar was prepared by dissolving 51.5 g of the powder in 1.0 L of distilled water, autoclave, according to manufacturer (De la Maza et al., 2013).

3.2.1.4. Kligler Iron agar

The Kligler Iron agar was prepared by dissolving 57.5 g in 1.0 L of distilled water, autoclave, according to manufacturer. It is contained glucose, lactose, and phenol red. It is an excellent medium for detecting glucose and lactose fermentation and it's contained iron salts that react with H_2S to form a dark precipitate of Iron sulfide. This medium is used in slants (De la Maza et al., 2013).

3.2.1.5. Simmons Citrate agar

The Simmons Citrate agar was prepared by dissolving 24.28 g in 1.0 L of distilled water, autoclave, according to manufacturer. This test was used to determine the ability of an organism to utilize sodium citrate as its only carbon source and inorganic ammonium salts as its only nitrogen source. Bacteria that can grow on this medium turn the green indicator to blue (De la Maza et al., 2013).

3.2.1.6. Indole Broth

The Indole broth was prepared by dissolving 15gm from peptone water in 1litter of distilled water, according to manufacturer, then sterilized by autoclave. This test was used to determine the ability of an organism to split tryptophan to form the compounds: indole, pyruvic acid and ammonia. Positive result change pink colored ring after addition Kovac's reagent (De la Maza et al., 2013).

3.2.1.7. Nutrient broth

The Nutrient broth was prepared by dissolving 13g from the powder in 1.0 L of distilled water, and autoclaved according to manufacturer.

3.2.1.8. Urea Agar Base

The Urea agar base was prepared by dissolving 24g from the powder in 950 mLof distilled water and 50 mL of uria solution 40%, and autoclaved according to manufacturer. This test was used to determine the ability of an organism to produce the urease enzyme (De la Maza et al., 2013).

3.2.2. Stains and indicators

3.2.2.1. Ethidium bromide stain

The Ethidium bromide was prepared by dissolving 0.1g of ethidium bromide powder in 1 mL.

3.2.2.2. Oxidase test Indicator

To determine the presence of bacterial cytochrome oxidase. The solution was prepared as 1% by dissolving 0.05 g of tetra methyl-p- phenylene diamine dihydrochloride in 5 mL distilled water.

3.2.3. Solutions and Buffers

3.2.3.1. 10X Tris-borate-EDTA buffer (TBE Buffer)

This Buffer consisting of 108g Tris-base,55g Boric acid and 0.5M EDTA (PH 8) 40 mL in 1.0 L of distill water. The TBE buffer was diluted into 1X by adding 900 mL of distill water to 100 mL of 10 X TBE buffer.

3.2.3.2. Loading dye 6X

The dye consist of loading dye and sodium dodecyl sulfate (SDS) solution.

3.2.4. Sample collection and cultivation

The isolates were isolated from 570 patients that were collected at the five diagnostic microbiology laboratories in Erbil/Iraq, Including, Al-Jumhory Hospital, Nanakali Hospital, Arzheen Private Hospital, Zheen International Hospitalf and King Private Laboratory. Inpatients and outpatients were included in this study. *A. baumannii* were isolated from different clinical sample (blood, urine, sputum, abscess, and wounds) from October 1st to December 31st, 2021. Then the samples were directly transferred onto blood agar and MacConkey agar, except for blood specimens which were directly transferred into a special blood culture bottle, followed by cultivation and incubation at 37 ˚C for 24 hours.

3.2.5. Storage of isolates

- For short term storage, the isolates were maintained by streaking them on surface of nutrient agar slants and incubated at 37˚C for 24 hours, then stored at 4˚C for one month.
- For long-term storage, the isolates were sub-cultured on MacConkey agar and incubated at 37°C for 24hrs, then several pure colony on the MacConkey agar are transferred into 1.5 ml Eppendorf tube which contains nutrient broth and mixed with 20 % glycerol, after that stored at -20˚C for 6 months or -80˚C for longer time (Forbes, 2007).

3.2.6. Identification of isolates

3.2.6.1. Phenotypic identification

3.2.6.1.1. Gram stain

A small portion of isolated colony was Gram stained on a slide and examined by light microscope; the following procedures are followed:

3.2.6.1.2. Conventional biochemical tests

3.2.6.1.2.1. Kligler iron agar

Kligler iron agar was inoculated by stabbing well isolated colony grown on MacConkey agar for 24 hours at 37˚C through the center of the medium to the bottom of the tube and then streaking the surface of the agar slant; the cap was leaved on loosely and incubated the tubes at

37˚C for 24 hours. K (alkaline) / A (acid) with H_2S (black precipitate) or without, bulb means producing gas.

3.2.6.1.2.2. Urease test

In this test the surface of Urea agar slant was streaked with a portion of a well isolated colony grown on MacConkey agar for 24 hours at 37˚C, and incubated at 37˚C for 24 hours. The color of slant is changed into pink, that is indicate positive result for *A. baumannii.*

3.2.6.1.2.3. Citrate test

Simmons Citrate agar slant were inoculated with a well isolated colony grown on MacConkey′s agar for 24 hours at 37˚C. Positive result indicated by color change from green to blue. The result is positive for *A. baumannii.*

3.2.6.1.2.4. Indole test

Peptone water was inoculated with a well colony grown on MacConkey′s agar for 24 hours at 37˚C, and incubated at 37˚C for 48 hours, then 0.5mL of Kovacs reagent was added. Positive result indicated by formation of pink to wine colored ring after addition appropriate reagent. *A. baumannii* is non indole former that it shows no colored change after addition reagent.

3.2.6.1.2.5. Oxidase Test

To a filter paper moistened with the substrate (1% tetramethyl-p-phenylenediamine dihydrochloride), with a wooden stick, a small portion of a colony grown on MacConkey′s agar for 24 hours at 37˚C was transferred to the moisted filter paper, the color of the paper change to deep blue or purple within ten seconds indicated positive result.

3.2.6.1.2.6. Catalase

Added a drop of hydrogen peroxide (H_2O_2) onto the clean glass slide and then used sterile wooden stick to transfer a small portion of a colony grown on MacConkey′s agar for 24 hours at 37˚C, into the H_2O_2 on the slide, positive result appeared after bulbs made in a few seconds.

3.2.6.1.3. Identification the isolated by using VITEK® 2 Compact system

3.2.6.1.3.1. Inoculum preparation

Suspensions were prepared by emulsifying bacterial isolates in saline to the equivalent of a 0.5 McFarland turbidity standard. The same suspension was used for identification (ID) and antimicrobial susceptibility test (AST) for the VITEK® 2 Compact system by using ID card and AST card. Suspensions for the comparative identification method were made according to the manufacturer's recommendations.

3.2.6.1.3.2. Biochemical analysis of bacterial isolates by using VITEK® 2 Compact system

Biochemical identification was performed using the VITEK® 2 Compact system with GN-ID cards automatic identification instrument for microorganisms, which is available in all microbiological laboratories. The GN-ID cards contain biochemical tests, including sugar assimilation, sugar fermentation, carbon source utilization, decarboxylase tests and enzymatic activities. The latest technology using VITEK® 2 Compact system makes it easy to use, namely with only 3 stages of examination that will easily obtain the results of identification and sensitivity of antibiotics that have been validated and interpreted by international standards (Clinical Laboratory Standard International (CLSI)) (Barry *et al.*, 2003; Larone, Tucci and Samide, 2000; Rombot and Y Semuel, 2021). The three stages are preparation or standardization of the inoculum turbidity, entering data with a barcode system, and inserting a card into the instrument. Furthermore, the whole process of inoculation, incubation, reading, validation, and interpretation of the results is be carried out automatically by the instrument. Furthermore, the completed examination is automatically producing a printout, while the Identification/ Antimicrobial Sensitivity Test (ID / AST) card by the system will automatically be discarded. The principle of automatic identification is to use an identification card. On the card, there is a well or like a biochemical test medium that is modified in such a way that it can be used for rapid identification of bacteria. The test procedure with the VITEK2 system tool starts from the gram test, selecting the card, and making a bacterial suspension according to the McFarland standard and identification using the tool until an identification result sheet comes out. Based on the theory that the results obtained in identification with VITEK® 2 Compact system are expressed as a percentage for the correctness of the identified organisms (Barry *et al.*, 2003; Rombot & Y Semuel, 2021)

3.2.6.2. Molecular identification:

3.2.6.2.1. DNA extraction

3.2.6.2.1.1. Extraction genomic DNA for PCR by using kit

The bacteria DNA extraction kit (POUYA GENE AZMA CO.) was employed as follows:

1. 1.5 mL of bacterial culture was collected in a microtube followed by centrifugated at 13000 rpm for 3 minutes.
2. The precipitate was re-suspended in 100µLBuffer I.
3. 200 µL Buffer II was added to the tube and gently inverted 3-5 times.
4. Then 150 µL Buffer III was added to the tube and gently inverted 3 times and kept at 37 C for 45 minutes.
5. Followed by adding 180 µL Buffer X to the tube and inverting 10 times. (When buffer III was added into the tube, a white precipitate was produced).
6. The tubes were centrifuged at 13000 rpm for 10 minutes and the supernatants were transferred into a new tube.
7. Then 2 volumes of cool ethanol %96 - %100 were added to the solution and gently inverted 5 times.
8. The tubes were micro-centrifuged at 13000 rpm for 5 minutes, and ethanol was discarded by gently inverting of the tube and keeping the precipitate.
9. The precipitate was washed by adding 700µL cool ethanol %70 and inverting 2-3 times.
10. Repeated step (8) and the precipitate was dried at room temperature for 1-2 minutes.
11. According to the precipitate, 20- 50 µL of solvent buffer (elution buffer) was added to the tube.
12. Finally, the DNA was stored at -20 ^{0}C for PCR purpose.

3.2.6.2.1.2. Extraction genomic DNA by boiling:

Following overnight sub-culture on MacConkey agar at 37°C, DNA extraction was performed by boiling few identical colonies in 100 µL of sterile distilled water for about 15-20 minutes. The Eppendorf tubes were centrifuged and the supernatant was used as the DNA template. The DNA was stored at -20 ^{0}C until used for PCR purposes. For use in PCR, the DNA was diluted in sterile nuclease free water or elution buffer to a concentration of 20-100 ng/µL.

3.2.6.2.2. Measuring concentration and purity of extracted DNA

The Thermo Scientific Nano drop spectrophotometer was used to verify the concentration and purity of the extracted DNA. An A260/A280 of 1.8-2.00 was considered suitable for DNA sequencing. For use in PCR, the DNA was diluted in sterile distillated water or elution buffer to a concentration of 20-100 ng/µL.

3.2.6.2.3. Gel electrophoresis

Gel preparation and electrophoresis were carried out as follows:

1. Agarose gel 1% was prepared by dissolved 1g agarose powder in 100mL 1X Tris-borate-EDTA buffer (TBE buffer)
2. The solution was microwaved in oven until the agarose was completely melted.
3. Then allowed to cool down to about 40°C to add ethidium bromide or safe dye to create the gel.
4. The comb was fixed at one end of the tray for making wells used for loading DNA samples.
5. The agarose solution was poured gently into the tray, and allowed to solidify at room temperature for 30 min.
6. After solidified, the comb was removed gently from the tray.
7. The gel was then placed in the gel electrophoresis box and TBE buffer solution was poured onto it.
8. The loading buffer 6X (dye) 1 was used to stain the DNA and make it thicker, or PCR product (amplified DNA) was inserted into the holes instead of the DNA template.
9. 1µl of loading dye 6X was added to 5 µl DNA template and inserted into the holes using a micropipette.
10. As well as a DNA ladder which already contains a loading buffer was used to measure the size of the DNA templates or amplified gene.
11. The electrical current was then turned on, and 75 voltages used for an hour. The DNA was placed in negatively charged side and the opposite side was positive due to the negative phosphate backbone of the DNA structure pulling it towards the positive side. The DNA repels the negative charge initiating movement.

12. The gel was removed and placed on the UV Viewing Cabinet, then the transilluminator was turned on and photographed.

3.2.6.2.4. Amplification of genes by Polymerase chain reaction (PCR)

All the PCRs were performed in a total volume of 25 µL, with reagents according to the manufacturer's guidelines. All the primers used in this study were from previously published papers, which are listed in table 3.6.

3.2.6.2.4.1. Molecular identification of *Acinetobacter baumannii* by using 16S and 23S rRNA gene (ITS) region

3.2.6.2.4.2. Amplification of ITS region and nucleotide sequence determination

The bacterium specific universal primers 1512F (5GTCGTAACAAGGTAGCCGTA3) and 6R (5GGGTTYCCCCRTTCRGAAAT3) (where Y is C or T and R is A or G) were used to amplify a DNA fragment that encompassed a small fragment of the 16S rRNA gene region, the ITS, and a small fragment of the 23S rRNA gene region. The 5 end of primer 1512F is located at position 1493 of the 16S rRNA gene, and the 5 end of primer 6R is located at position 108 downstream of the 5 end of the 23S rRNA gene (*Escherichia coli* numbering). PCR was performed with 2 µL of template DNA in a total reaction volume of 25 µL, 12.5 µL of master mix consisting of 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.8 mM deoxyribonucleoside triphosphates (0.2 mM each), 1 U of Taq DNA polymerase, 1 µL of each primer and then completed with free DNase water to 25 µL. The PCR program consisted of an initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation (94°C for 1 min), annealing (51°C for 1 min), and extension (72°C for 1 min), with a final extension step at 72°C for 7 min. An OmniGen thermal cycler was used for PCR (Chang et al., 2005).

3.2.7. Quantification of Biofilm Formation:

Polystyrene microtiter plate (M.T.P) based on the safranin staining method was applied as follows with few modification in the procedure (Bordeleau et al., 2018).

- Nutrient broth was prepared and supplemented with 2% (w/v) glucose.
- Then the medium was inoculated with the *A. baumannii* strains and after that incubated overnight at 37 ^{0}C.

- To each well of M.T.P, 180µl of normal saline was added followed by adding 20 µl of the overnight culture bacteria and incubated for 37 C for 24 h (three wells used to each strain).
- The media was discarded and the wells were gently washed twice with 200 distilled water and dried.
- Each well was stained with 200 µl of 0.1% safranin and incubated for 10 min at room temperature.
- Each well was washed 3 times with 200 µl of distilled water and allowed to dry completely at room temperature.
- Finally, the optical density (OD) for each well was measured at 490 nm using BioTic ELISA reader (ELx800).
- Wells to which sterile broth without of bacteria was included and served as a negative control; OD values for these wells were subtracted from the experimental readings.

3.2.7.1. Cut-off value calculation

The optical density cut-off value (ODc) was measured and biofilm formation was detected using the formula described by (Bardbari et al., 2017). The assay was done at triplicate for each isolate.

The optical density cut-off value (ODc) was measured by the following formula:

ODc = mean of negative control OD + (3 × negative control SD).

OD is optical density

SD is standard deviations

The results were classified into four groups depending to their optical densities as

(1) strong biofilm formation (4 × ODc and lt; OD)

(2) medium biofilm formation (2 × ODc and lt; OD ≤ 4 × ODc)

(3) weak biofilm formation (ODc and lt; OD ≤ 2 × ODc)

(4) non-biofilm formation (OD ≤ ODc)

3.2.8. Plasmid DNA extraction

High-Speed Plasmid mini kit used included these steps:

1. 1.5 ml of overnight cultured bacterial cells was transferred to a microcentrifuge tube, and centrifuged at 12,000 rpm for 1 minute then the supernatant was discarded.
2. Step 1 Re-suspension: 200 µL of PD1 Buffer (RNase A was added) was added to the tube, and re-suspended the cell pellet by vortex.
3. Step 2 Lysis: 200 µL of PD2 Buffer was added (all precipitates were dissolved), and the tube was mixed gently by inverted 10 times without vortex to avoid shearing the genomic DNA and left at room temperature for at least 2 minutes.
4. Step 3 Neutralization: 300 µL of PD3 Buffer was added to the tube and mixed immediately by inverting the tube 10 times without vortex to avoid shearing the genomic DNA, and centrifuged at 12,000 rpm for 3 minutes.
5. Step 4 DNA Binding. a PD column was placed in a 2 mL collection tube and the supernatant from Step 3. was added with centrifuged at 12,000 rpm for 30 seconds then the flow-through was discarded and the PD Column was placed back in the 2 mL collection tube.
6. Step 5 Wash Optional: 400 µL of W1 Buffer was added into the PD Column, followed by centrifuging at 12,000 rpm for 30 seconds then discarding the flow-through.

   The PD Column was placed back in the 2 mL collection tube and proceeded with the addition of wash buffer. Then 600 µL of wash buffer (ethanol was added) was into the PD Column and centrifuged at 12,000 rpm for 30 seconds.

   The flow through was discarded and the PD Column was placed back in the 2 mL collection tube, centrifuged at 12,000 rpm for 3 minutes to let the column matrix dry, the PD Column was placed in a new 1.5 mL microcentrifuge tube.
7. Step 6 DNA Elution: for higher DNA concentration used 30 µL of Elution Buffer, whilst for maximum DNA yield used 100 µL of Elution Buffer (DNA concentration would dilute).

   Then, 50 µL of Elution Buffer or TE was added into the center of the PD Column matrix, and left for at least 2 minutes to allow the Elution Buffer or TE to be completely absorbed followed by centrifuged at 12,000 rpm for 2 minutes to elute the purified DNA.

A second elution step was performed to increase the yield between 10-30%. The flow-through was transferred back into the center of the PD Column matrix, and centrifuged at 12,000 rpm for 2 minutes to elute the purified DNA.

The gel electrophoresis and the Thermo Scientific Nanodrop spectrophotometer were used to verify the concentration and purity of the extracted DNA. An A260/A280 of 1.8-1.9 was considered suitable for DNA sequencing.

3.2.9. Antibiotic resistance

3.2.9.1. Phenotypic detection

3.2.9.1.1. Detection of Antibiotic Susceptibility by using VITEK® 2 Compact system

Susceptibility tests with the VITEK® 2 Compact system was performed with AST-N326 cards, according to the manufacturer's instructions (section 3.2.5.1.3.). AST-N326 (bioMérieux) card included piperacillin (PIP), ceftazidime (CAZ), aztreonam (ATM), levofloxacin (LEV), cefepime (FEP), trimethoprim/sulfamethoxazole (SXT), ciprofloxacin (CIP), tazobactam/ piperacillin (TZP), netilmicin (NET), gentamicin (GEN), amikacin (AK), imipenem (IPM), meropenem (MEM) and tigecycline (TIG). The cards were filled with an inoculum of 1.5×10^8 CFU/ml (prepared from the 0.5 McFarland suspension used with the identification cards) and then sealed and read. The VITEK 2 system automatically processed the antimicrobial susceptibility cards until the MICs are obtained. The results were interpreted using VITEK 2 software version 08.01, and final results were obtained automatically.

3.2.9.2. Genotypic detection

3.2.9.2.1. Molecular detection of the antibiotic resistance genes in *Acinetobacter baumannii*

- The genomic DNA and plasmid DNA of all isolates were included to detect the resistance genes. Twenty-Two pairs of primer were used and each primer pair detected a specific resistance gene of each isolate (table 3.6).
- The PCR was performed in a total volume 25 µl; 2 µl of the DNA template isolates with 12.5µl of master mix and 1µl of each primer and then completed the volume by double distillated water.
- For multiplex PCR, 2 µl of each primer, 4 µl of DNA template and 25 µl of master mix used in 50 µl total volume and completed the volume by double distillated water.
- All amplification conditions for each primer clarified in (table 3.7).

3.2.9.2.2. Antibiotic resistance genes

Resistance genes of the isolates were determined in both DNA chromosome and plasmid by PCR amplification.

The isolate's resistance gene detection was performed as follows:

i. OXA-type β-lactamases genes were identified and followed by sequences of the PCR product.

Class A was bla_{SHV} and bla_{TEM} genes,

Class B was bla_{NDM} gene,

Class D was bla_{OXA-23}, bla_{OXA-27}, bla_{OXA-49}, bla_{OXA-58}, bla_{OXA-96}, bla_{OXA-51}, bla_{OXA-58}, bla_{OXA-64}, bla_{OXA-69}, bla_{OXA-70}, bla_{OXA-71}, bla_{OXA-75}, and bla_{OXA-78}.

ii. Detection of the resistance genes performed for the following antibiotics:

Streptomycin *strA* and *strB*

Tetracycline *tetA* and *tetB*

Fluoroquinolone *gyrA, parC*

Sulfonamides *sul I*, *sul II*

Macrolides *mph(E), msr(E)* and *erm 42*

Efflux pumps conferring antibiotic resistance *adeB*

aminoglycoside *aph(3')-VI, aacA4*

trimethoprim *dhfr1*

aadB, confers tobramycin, gentamicin, and kanamycin resistance.

DNA sequence was performed for both strands (primers forward and reverse) by the Immuno Gene Center and the DNA sequence was analyzed by used Bio-Edit program software. Online similarity searching was performed with the BLAST (Basic Local Alignment Search Tool) family of programs in GenBank.

Table 3. 5. Primers used in the study:

Primer	Target genes	Sequence (5-3)	Amplicon size (bp)	References
1512F	16S-23S rRNA	GTCGTAACAAGGTAGCCGTA	790	(Chang et al.,

6R		GGGTTYCCCCRTTCRGAAAT3		2005)
OXA 51 F	$bla_{\text{OXA-51-like}}$	TAATGCTTTGATCGGCCTTG	320	(Turton et al., 2006)
OXA 51 R		TGGATTGCACTTCATCTTGG		
OXA 58 F	$bla_{\text{OXA-58}}$, $bla_{\text{OXA-96}}$	ATGAAATTATTAAAAATATTG AGTTTAG	≈500	(Hujer et al., 2006a)
OXA58 R		TTATAAATAATGAAAAACACC CAAC		
OXA 23 F	$bla_{\text{OXA-23-like}}$	ACAGAARTATTTAAGTGGG	350	
OXA 23 R		GGTCTACAKCCMWTCCCCA		
NDM F	bla_{NDM}	GGTTTGGCGATCTGGTTTTC	590	(Poirel, Walsh, et al., 2011)
NDM R		CGGAATGGCTCATCACGATC		
SHV F	bla_{SHV}	ATGCGTTATATTCGCCTGTG	≈200	(Hujer et al., 2006a) 41
SHV R		TGCTTTGTTATTCGGGCCAA		
TEM F	bla_{TEM}	AAACGCTGGTGAAAGTA	720	
TEM R		AGCGATCTGTCTAT		
aacA4 F	*aacA4*	ATGACTGAGCATGACCTTGCG	450	
aacA4 R		TTAGGCATCACTGCGTGTTCG		
aadB F	*aadB*	ATGGACACAACGCAGGTCGC	≈219	
aadB R		TTAGGCCGCATATCGCGACC		
aph(3')-VI F	*aph(3')-VI*	CGGAAACAGCGTTTTAGA	716	(Noppe-Leclercq et al., 1999)
aph(3')-VI R		TTCCTTTTGTCAGGTC		
AdeB F	*adeB*	CTTGCATTTACGTGTGGTGT	200	(Modarresi, *et al.*, 2015)
AdeB R		GCTTTTCTACTGCACCCAAA		
gyrA F	*gyrA*	AAATCTGCCCGTGTCGTTGGT	≈350	(Hujer et al., 2006a)
gyrA R		GCCATACCTACGGCGATACC		
ParC F	*parC*	AAACCTGTTCAGCGCCGCATT	327	
parC R		AAAGTTGTCTTGCCATTCA		
dhfr1 F	*dhfr1*	CGGTCGTAACACGTTCAAGT	220	(Cho et al., 2019)
dhfr1 R		CTGGGGATTTCAGGAAAGTA		
msr(E) F	*msr(E)*	TATAGCGACTTTAGCGCCAA	395	(Rose et al., 2012)
msr(E) R		GCCGTAGAATATGAGCTGAT		
mph(E) F	*mph(E)*	ATGCCCAGCATATAAATCGC	271	
mph(E) R		ATATGGACAAAGATAGCCCG		
erm(42) F	*erm(42)*	TGCACCATCTTACAAGGAGT	173	
erm(42) R		CATGCCTGTCTTCAAGGTTT		
strA F	*strA*	GGTAAGAAGTCGGGATTGAC	165	(Brunelle et al., 2015)
strA R		CACAGCCTATCGGTTGATC		
strB F	*strB*	CGCCATACCAGATAGTCG	600	
strB R		CTTTTCCAGCCTCGTTTG		
sul I F	*sul I*	CGGCGTGGGCTACCTGAACG	433	(Kerrn et al., 2002)
sul I R		GCCGATCGCGTGAAGTTCCG		
sul II F	*sul II*	GCGCTCAAGGCAGATGGCATT	293	
sul II R		GCCACTGCGCCGTTACCACC		
tetA F	*tetA*	GCGCGATCTGGTTCACTCG	164	(Aminov et al., 2002)
tetA R		AGTCGACAGYRGCGCCGGC		
tetB F	*tetB*	CGTTTGCTTTCAGGGATCA	437	(Stine et al., 2007)
tetB R		ACCATCATGCTATTCCATCC		

Table 3. 6. The primers and their condition protocols

No.	primer	No. of cycles	Initial denaturation	Time mints	Denaturation	Time second	Annealing temp.	Time second	Extension	Time second	Final extension	Time mints
1	16S 23S rRNA	35	94	2	94	60	51	60	72	60	72	7
2	OXA 51	30	95	3	95	45	57	45	72	60	72	5
3	OXA 58	30	94	10	94	30	53	60	72	50	72	5
4	OXA SET B	30	94	10	94	30	51	60	72	50	72	5
5	OXA SET C	30	94	10	94	30	47	60	72	50	72	5
6	TEM	30	94	10	94	30	45	60	72	50	72	5
7	SHV	30	94	10	94	30	60	60	72	50	72	5
8	NDM	30	95	5	95	30	52	30	72	45	72	5
9	aacA4	30	94	10	94	30	65	60	72	50	72	5
10	aadB	30	94	10	94	30	68	60	72	50	72	5
11	aph(3') VI	35	94	3	94	30	49	40	72	60	72	5
12	adeB	30	95	2	95	60	54	45	72	60	72	10
13	msr(E)	25	95	5	94	30	68	30	72	45	72	5
14	mph(E)	25	95	5	94	30	68	30	72	45	72	5
15	Erm(42)	25	95	5	94	30	68	30	72	45	72	5
16	parC	30	94	10	94	30	58	60	72	50	72	5
17	gyrA	30	94	10	94	30	63	60	72	50	72	5
18	strA	35	94	1	94	30	56	30	72	30	72	2
19	strB	35	94	1	94	30	56	30	72	30	72	2
20	sul I	30	94	5	94	15	69	30	72	60	72	7
21	sul II	30	94	5	94	15	69	30	72	60	72	7
22	Tet A	25	95	5	94	50	61	30	72	30	72	7
23	Tet B	25	95	5	94	50	61	30	72	30	72	7
24	dhfr1	30	94	2	94	60	54	45	72	60	72	10

3.2.10. Statistical analysis

Using the GraphPad Prism (Version.9) software and T-test to compare the means of tests and controls using a computer program of epidemiological statistics and the value was regarded significant if $P \leq 0.05$.

3.2.11. Phylogenetic analysis for novel genes

MEGA11 software program was used building a phylogenetic tree for novel genes in current study.

Chapter Four

4. Results

4.1. Samples collection

The specimens included in the study were urine (catheterized and non-catheterized), sputum (expectorated sputum and endotracheal aspirates) blood, stool, pus, swab, and fluid tables (4.1). Out of 570 samples, (100) samples were Covid19 patients including 13 of them (13%) were coinfected with *A. baumannii*.

Table 4. 1. Types of the samples used in this study

No.	Type of the specimens	Number of the sample (%)	Number of isolates (%)
1	Sputum	93 (16.32%)	16 (17.2%)
2	Urine	355 (62.28%)	7 (1.97%)
3	Blood	43 (7.54%)	0
4	Stool	16 (2.80%)	0
5	Pus	13 (2.28%)	0
6	Swab	38 (6.67%)	0
7	Body fluid	12 (2.12%)	0

4.2. Isolation and identification of the clinical isolates

Among the 570 specimens, 23 (4.04%) were identified as *A. baumannii* (table 4.1) based on the colony morphology, Blood agar, and MacConkey agar, microscopically (gram stain), biochemical tests by using VITEK® 2 Compact system (tables 4.2), manual biochemical tests. However, 17 isolates involving all the 13 Covid 19 which coinfected with *A. baumannii* were used in this study.

4.2.1. Colony morphology

4.2.1.1. MacConkey agar

A selective and differential medium used to test the isolate's ability to ferment lactose. After cultivation of isolates on MacConkey agar at 37 C for 24 hours the colonies of *A. baumannii* appeared as non-lactose fermenting (pale color) or partial lactose fermenting (light

pink), small arranged 1 to 3 millimeters, mucoid, smooth, pale color, raised or domed and round (figure 4.1).

4.2.1.2. Blood agar

Blood agar was prepared by adding %5 of human blood and used to detect *A. baumannii* ability to hemolysis red blood cells. The colonies appeared small 1-2 millimeters, translucent to opaque, convex, rounded, smooth surface, entire margins colonies, creamy and non-hemolytic colonies when cultured on blood agar medium because of the disability of this bacterium to produce hemolysins. Hemolysins are responsible for breaking down red blood cells by destroying their cell membrane (figure 4.1).

4.2.2. Microscopic examination (Gram staining)

All the examined isolates appeared as gram-negative coccobacilli and sometimes they organized as diplococci, (figure 4.1).

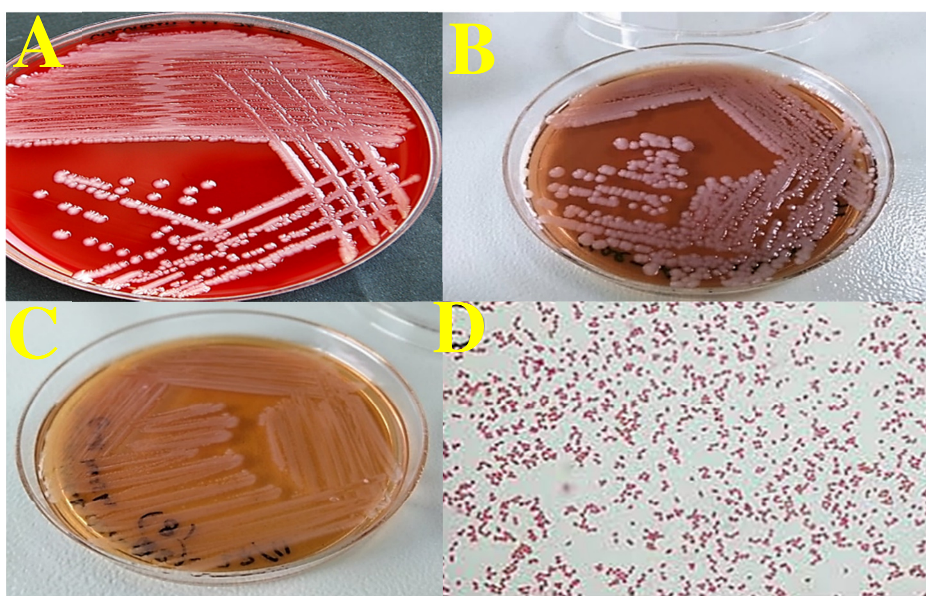

Figure 4.1. Colony morphology of *A. baumannii* culture., A: *A. baumannii* colony on Blood agar, B: the partial lactose fermenting *A. baumannii*, C: is the non-lactose fermenting *A. baumannii* and D: is *A. baumannii* stained with gram stain under microscope.

4.2.3. Biochemical tests

The isolates which were cultivated and grown on MacConkey agar showed gram-negative bacteria then to confirm the results, several biochemical tests were used to identify the isolates. Oxidase, catalase, indole, citrate utilization, Kligler iron agar and urease tests were used.

- All isolates were urease positive test for urease test, it showed the ability of urease enzyme to analysis urea and produce ammonia.
- The same result was demonstrated for catalase and citrate utilization test, the catalase test related to the ability to break hydroxide in water which causes the release of oxygen as bubbles, and citrate utilization test showed the color of simmon citrate media converted to blue which demonstrated using sodium citrate as a carbon source.
- The result of Kliger iron agar showed alkaline in both slant and bottom, no H_2S and no gas production in 16 isolates (94.12%), except in one isolate (5.88%) showed production of H_2S and gas. The lack of tryptophanase enzyme prevents the isolates to hydrolyze tryptophan and convert it into indole, all isolates were negative to indole test.
- Positive results were observed for oxidase test in 16 isolates (94.12%), one (5.88%) of the isolates showed a negative result.

4.2.3.1. Biochemical test results by VITEK® 2 Compact system *Acinetobacter baumannii*

The result for all isolates were 99% probability reported as *A. baumannii* is represented in appendix 11 and 12.

4.2.4. Molecular identification

4.2.4.1. Genotypic identification of *Acinetobacter baumannii* isolates by detecting ITS gene fragment (16s & 23s rRNA gene) and sequencing

Figure 4.2 shows PCR amplification results for 16S & 23S rRNA gene (ITS) with primers 1512F and 6R for 17 isolates. However, the sequencing analysis of ITS amplified fragments of 9 isolates (those PCR products that appeared as a single band on gel electrophoresis) resulted, 7 isolates were *A. baumannii*, one isolate was *Pseudomonas aeruginosa* and one isolate identified as *Stenotrophomones maltophilia* novel strain submitted to GenBank under accession number OP422244. The accession numbers are listed in (table 4.6 and Appendix 5). However, 8 isolates PCR products had double band on gel electrophoresis, one of these isolates that had double band on gel electrophoresis was used to sequencing the ITS amplified fragments, and the result was not good and reliable. These isolates identified as *A. baumannii* with the VITEK® 2 Compact system, biochemical tests and β-lactamases genes sequencing analysis (table 4.6).

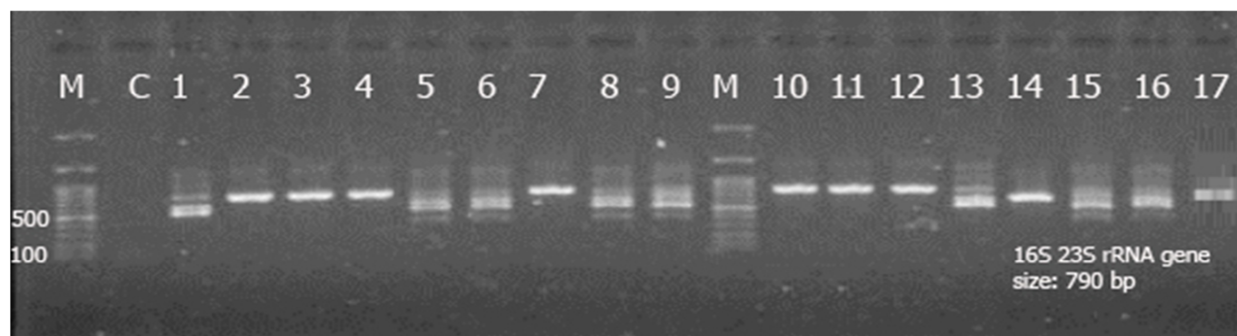

Figure 4.2. Gel electrophoresis of PCR products for gene 16S 23S rRNA (790 bp) for identification of *A. baumannii*. Lane M is 100 bp DNA ladder and lane 1-17 positive amplicon size for gene 16S 23S rRNA.

4.2.4.2. Bacteria characterization

4.2.5. Biofilm formation

The bacterial isolates were variable to produce biofilm; moderate biofilm formation was detected in 7 (41.2%) isolates. Weak biofilm formation was detected in 6 (35.3%) isolates, and non-biofilm formation in 3(17.6%) isolates. However, the non-biofilm formation isolates were statistically non-significant (table 4.2).

Table 4.2. Pattern of biofilm formation among the bacterial isolates.

Strains	OD at 490 nm (mean ± SD)	Biofilm ability formation	P-value	t-test	Standard Error (SD)	Statical significance
1	0.05033 ±0.00107	Non- biofilm formation	0.9845	0.0206	0.016	Non- significant
2	0.07767 ±0.0101	Weak biofilm formation	0.1794	1.6255	0.017	Non- significant
3	0.1877 ±0.0535	Medium biofilm formation	0.0167	3.9588	0.035	significant
4	0.1347 ±0.0335	Medium biofilm formation	0.0279	3.3749	0.025	significant
5	0.1433 ±0.0117	Medium biofilm formation	0.0058	5.3742	0.017	Very significant
6	0.1527 ±0.0477	Medium biofilm formation	0.0321	3.2249	0.032	significant

7	0.08933 ±0.0182	Weak biofilm formation	0.109	2.0553	0.019	Non- significant
8	0.05333 ±0.00107	Non- biofilm formation	0.8453	0.2081	0.016	Non- significant
9	0.1443 ±0.00786	Medium biofilm formation	0.0048	5.6725	0.017	Very significant
10	0.063 ±0.0037	Weak biofilm formation	0.4656	0.8057	0.016	Non- significant
11	0.1493 ±0.0246	Medium biofilm formation	0.0097	4.6426	0.021	Very significant
12	0.1207 ±0.0252	Medium biofilm formation	0.0308	3.2701	0.022	significant
13	0.057 ±0.00333	Non- biofilm formation	0.6863	0.4346	0.016	Non- significant
15	0.06633 ±0.00324	Weak biofilm formation	0.3679	1.0142	0.016	Non- significant
17	0.1027 ±0.00656	Weak biofilm formation	0.0327	3.2066	0.016	significant
(14) *P. aeruginosa*	0.073 ±0.00733	Weak biofilm formation	0.2368	1.3903	0.017	Non- significant
Control	0.05 ±0.00277					

*≤ 0.23 is strong biofilm formation

= 0.12 is medium biofilm formation

from 0.06 to 0.12 is weak biofilm formation

≥ 0.06 is non-biofilm formation

4.2.6. Plasmid detection

All isolates carried a plasmid, the purity of DNAs ranged ratio of 1.8-2.00 by using nanodrop (figure 4.3), and the concentrations of the plasmid DNAs ranged between 20 to 100 ng/ul.The DNAs were used to determine resistance genes.

Plots Report | Test type: Nucleic Acid | Exit

Report Name | Report Full Mode

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
1	Default	1/1/2002	4:40 AM	36.15	0.723	0.388	1.86	1.18	50.00	230	0.615	0.190
2	Default	1/1/2002	4:41 AM	35.80	0.716	0.366	1.96	1.52	50.00	230	0.472	0.073
3	Default	1/1/2002	4:42 AM	50.88	1.018	0.508	2.00	1.82	50.00	230	0.561	0.108
4	Default	1/1/2002	4:42 AM	37.89	0.758	0.342	1.79	2.09	50.00	230	0.362	0.009
5	Default	1/1/2002	4:43 AM	95.26	1.905	1.033	1.84	1.15	50.00	230	1.652	0.684
6	Default	1/1/2002	4:44 AM	125.66	2.513	1.212	2.00	2.08	50.00	230	1.207	0.093
7	Default	1/1/2002	4:44 AM	13.34	0.267	0.141	1.89	1.03	50.00	230	0.260	0.231
8	Default	1/1/2002	4:45 AM	32.04	0.641	0.350	1.83	0.98	50.00	230	0.653	0.310
9	Default	1/1/2002	4:46 AM	110.77	2.215	1.097	2.00	2.14	50.00	230	1.035	0.091
10	Default	1/1/2002	4:46 AM	40.76	0.815	0.508	1.79	0.65	50.00	230	1.251	0.646
11	Default	1/1/2002	4:47 AM	124.46	2.489	1.578	1.86	0.65	50.00	230	3.806	2.552
12	Default	1/1/2002	4:47 AM	39.75	0.795	0.408	1.95	1.73	50.00	230	0.458	0.075
13	Default	1/1/2002	4:48 AM	38.91	0.778	0.420	1.85	1.05	50.00	230	0.738	0.250
14	Default	1/1/2002	4:49 AM	42.59	0.852	0.459	1.86	1.35	50.00	230	0.631	0.102
15	Default	1/1/2002	4:49 AM	92.84	1.857	1.023	1.82	1.41	50.00	230	1.316	0.346
17	Default	1/1/2002	4:50 AM	50.24	1.005	0.523	1.92	1.61	50.00	230	0.625	0.099

Figure 4. 3. Nanodrop results of plasmid DNA extracted from isolates.

4.2.7. Antibacterial susceptibility detection

4.2.7.1. Phenotypic detection

The VITEK® 2 Compact system was used to screen and determine the MIC of antibacterial agents (table 4.3). All the isolates, 17 (100%), were resistant to Piperacillin, Piperacillin /tazobactam, ceftazidime, and levofloxacin. In contrast, 17 (100%) isolates were sensitive to colistin. However, 16 (94.1%) isolates were shown resistance to cefepime, tetracycline, imipenem, and ciprofloxacin, and 1 (5.9%) was shown susceptible results for these antibiotics. Besides, 15 (88.2%) were resistant, and 2 (15.8%) were susceptible to meropenem. On the other hand, 14 (82.4%) were resistance isolates, and 3 (17.6%) sensitive isolates were found to trimethoprim/ sulfamethaxole, 11 (58.8%) of the isolates were revealed as resistance, 6(35.3%) as susceptible and 1 (5.88) as intermediate to netilmicin. As well, gentamicin test results were 10 (58.8%) resistance isolates, 6 (36.4) susceptible isolates, and 1 (5.9%) intermediate. For tobramycin antibiotic, 9 (53%) were resistant and 8 (47%) as susceptible, and tigecycline antibiotic results showed 1 (5.9%) isolate resistant, 14 (82.4%) intermediate, and 2 (11.7%) susceptible (table 4.3).

Table 4. 3. Antibiotics susceptibility VITEK® 2 Compact system against *A. baumannii* isolates.

antimicrobial agent (no. of isolates tested)	MIC (µg/mL)		No. (%) of isolates		
	Range	No. (%) of isolates	Susceptible	Intermediate	Resistant

Piperacillin	≥ 128	17 (100)	0	0	17 (100)
Piperacillin/Tazobactam	≥ 128	17 (100)	0	0	17 (100)
ceftazidime	≥ 64 ≥ 32	15 (88.2) 2 (11.8)	0	0	17 (100)
cefepime	1 16 ≥ 32 ≥ 64	1(5.9) 1(5.9) 12 (70.6) 3 (17.6)	1(5.9)	0	16(94.1)
imipenem	1 ≥ 16	1(5.9) 16(94.1)	1(5.9)	0	16(94.1)
meropenem	≤ 10 ≥ 16	2 (11.8) 15(88.2)	2(11.8)	0	15(88.2)
Gentamicin	≤ 1 8 ≥ 16	6(35.3) 1(5.9) 10(58.8)	6(35.3)	1(5.9)	10(58.8)
Netilmicin	≥ 32 16 4 2	11 (64.7) 1(7.7) 4 (30.8) 1(7.7)	5 (38.5)	1(7.7)	11 (64.7)
Tobramycin	≥ 16 4 2 ≤ 1	6 (42.9) 1 (7.1) 1 (7.1) 6 (42.9)	8 (47)	0	9 (53)
ciprofloxacin	≥ 4	16 (94.1)	1 (5.9)	0	16 (94.1)
levofloxacin	≥ 8	17 (100)	0	0	17(100)
tetracycline	≥ 16 4	13 (92.9) 1 (7.1)	1 (5.9)	0	16 (94.1)
tigecycline	≥ 8 4 2 ≥ 0.5	1 (7.7) 12 (61.5) 3 (23.1) 1 (7.7)	4 (23.5)	12 (70.6)	1 (5.9)
colstin	≥ 0.5	17 (100)	17(100)	0	0
trimethoprim/ sulfamethaxole	≥320 80 ≥20	13 (76.5) 1 (5.9) 3 (17.6)	3(17.6)	0	14 (82.4)

4.2.7.2. Genotypic detection

4.2.7.2.1. Detection of β-lactamase (Extended-spectrum β-lactamases (ESBLs)) genes and sequencing:

Table 4.4 lists the percentage of detected β-lactamase genes in extracted chromosomal DNA and plasmid DNA. All the extracted chromosomal DNA undergoes detection of all β-lactamase genes. Therefore, the plasmid DNA isolates screened to ESBLs genes. The results that were found were interested. Some of these genes presented in both chromosomal DNA and plasmid DNA, and some of these genes were just found in chromosomal DNA or plasmid DNA of the isolates (table 4.4 and 4.5). The *bla*OXA-51 like gene found in 11 (64.70%) out of 17 isolates genomic DNA.

Table 4.4. The rate of β-lactamases gene detected in chromosomes and plasmids of *A. baumannii* Isolates

Genes encoding β-lactamases	% Detection in isolates chromosome DNA	% Detection in isolates plasmid
***bla**$_{OXA-51}$**-like**[1]*	58.8	17.64
***bla**$_{OXA-23}$**-like**[2]*	29.4	41.2
***bla**$_{OXA-58}$**-like**[3]*	0	17.6
***bla**$_{TEM}$*	100	0
***bla**$_{SHV}$*	0	23.5
***bla**$_{NDM}$*	76.5	47

[1] Includes *bla*$_{OXA-51-}$, *bla*$_{OXA-58-}$, *bla*$_{OXA-64-}$, *bla*$_{OXA-69-}$, *bla*$_{OXA-70-}$, *bla*$_{OXA-71-}$, *bla*$_{OXA-75-}$ and *bla*$_{OXA-78-like}$ genes.

[2] Includes *bla*$_{OXA-23-}$, *bla*$_{OXA-27-}$, and *bla*$_{OXA-49-like}$ genes.

[3] Includes *bla*$_{OXA-58-}$ and *bla*$_{OXA-96-like}$ genes.

Table 4. 5. *β-lactamases* gene detected in among 17 isolates of *A. baumannii.*

Isolates No.	**β- lactamases gene**											
	***bla**$_{OXA-51}$**-like**[1]*		***bla**$_{OXA-23-}$**like**[2]*		***bla**$_{OXA-58-}$**like**[3]*		***bla**$_{TEM}$*		***bla**$_{SHV}$*		***bla**$_{NDM}$*	
	Chromosome DNA	Plasmid DNA	Chromosome DNA	Plasmid DNA	Chromosome DNA	Plasmid DNA	Chromosome DNA	Plasmid DNA	Chromosome DNA	Plasmid DNA	Chromosome DNA	Plasmid DNA
1	-	+	-	+	-	-	+	-	-	-	-	+
2	+	-	+	+	-	-	+	-	-	-	+	-

3	+	-	+	+	-	+	+	-	-	-	-	+
4	+	-	+	+	-	+	+	-	-	-	+	+
5	+	-	+	-	-	+	+	-	-	+	+	-
6	-	-	-	-	-	-	+	-	-	-	-	+
7	+	-	+	-	-	-	+	-	-	-	+	-
8	+	-	-	-	-	-	+	-	-	-	+	+
9	-	+	-	-	-	-	+	-	-	-	+	-
10	+	-	-	-	-	-	+	-	-	+	+	-
11	+	-	-	+	-	-	+	-	-	+	+	+
12	+	-	-	+	-	-	+	-	-	-	+	+
13	-	-	-	+	-	-	+	-	-	-	+	-
14	-	-	-	-	-	-	+	-	-	-	+	-
15	-	-	-	-	-	-	+	-	-	+	+	+
16	-	-	-	-	-	-	Nil	-	Nil	-	-	-
17	+	+	-	Nil	-	-	+	-	-	-	+	-

[1] Includes *bla*$_{OXA-51-}$, *bla*$_{OXA-58-}$, *bla*$_{OXA-64-}$, *bla*$_{OXA-69-}$, *bla*$_{OXA-70-}$, *bla*$_{OXA-71-}$, *bla*$_{OXA-75-}$ and *bla*$_{OXA-78-like}$ genes.

[2] Includes *bla*$_{OXA-23-}$, *bla*$_{OXA-27-}$, and *bla*$_{OXA-49-like}$ genes.

[3] Includes *bla*$_{OXA-58-}$ and *bla*$_{OXA-96-like}$ genes.

Table 4.6. Molecular identification result by analysis and sequencing of ITS fragments amplification and β- lactamases genes of isolates

Isolates	***23S/16SrRNA***	**β- lactamases gene**			
		blaOXA-51-like	***blaOXA-23-like***	***blaNDM***	***blaTEM***
1					*A. baumannii* strain beta-lactam resistance HK22 [5]
2		*A. baumannii* strain Abau36 OXA-51 [2]	*A. baumannii*		
3	*A. baumannii*	*A. baumannii* strain Abau36 OXA-51 [2]	*A. baumannii*		
4	*A. baumannii*	*A. baumannii* strain Abau36 OXA-51 [2]	*A. baumannii*	*A. baumannii strain IPK-9 subclass B1 metallo-beta-lactamase NDM-42 (blaNDM)* [3]	*A. baumannii*
5		*A. baumannii* strain Abau36 OXA-51 [2]			
6	*A. baumannii*				*A. baumannii*
7	*A. baumannii*	*A. baumannii* strain Abau36 OXA-51 [2]	*A. baumannii*	*A. baumannii strain IPK-9 subclass B1 metallo-beta-lactamase NDM-42 (blaNDM)* [3]	
8	*A. baumannii*				*A. baumannii* strain beta-lactam resistance HK22 [5]
9				*A. baumannii* strain	*A. baumannii*

				carbapenems resistance HK19[4]	strain beta-lactam resistance HK22 [5]
10	*A. baumannii*	*A. baumannii* strain Abau36 OXA-51 [2]	*A. baumannii*	*A. baumannii strain IPK-9 subclass B1 metallo-beta-lactamase NDM-42 (blaNDM)*[3]	
11*	*A. baumannii*	*A. baumannii* strain Abau36 OXA-51[2]			*A. baumannii* strain beta-lactam resistance HK22 [5]
12	*A. baumannii*				
13					
14	*Pseudomonas aeruginosa*				
15					
16				*A. baumannii* strain carbapenems resistance HK19[4]	
17	*Stenotrophomones maltophilia* strain HK[1]		*Stenotrophomonas maltophilia strain HK OXA-23* [6]	*A. baumannii strain IPK-9 subclass B1 metallo-beta-lactamase NDM-42 (blaNDM),*[3,6]	

*the isolate was coinfected with *E.coli*.

[1] new strain in this study under accession number OP422244 in gene bank

[2] new OXA-51 family class D beta-lactamase from *A.baumannii* , submitted in 25-May-2022 French National Reference Center for antibiotics resistance, University Hospital of Besancon, France.

[3] novel gene submitted in 7/3/2022 in Japan.

[4] new strain of beta-lactamase (bla_{NDM}) gene in this study under accession number OP572243 in gene bank

[5] *A. baumannii* new strain of beta-lactamase (bla_{TEM}) gene in this study under accession number OP572244 in gene bank

[6] A new strain of *Stenotrophomonas maltophilia* strain HK *OXA-23* family carbapenem-hydrolyzing class D beta-lactamase (*blaOXA*) and bla_{NDM} gene in this study under accession numbers OP595162 and OP595163 in gene bank.

All 15 isolates of *A. baumannii* were carbapenemase production, however bla_{OXA58} and bla_{SHV} genes were not detected in chromosomel DNA (table 4.4 and 4.5). The presence of four β-lactamases genes (bla_{OXA23}-like genes, bla_{OXA-51}-like gene, bla_{TEM} and bla_{NDM}) were detected in 4 (26.7%) isolates, the 3 β-lactamases genes (bla_{OXA-51}-like gene, bla_{TEM} and bla_{NDM}) were detected in 5(33.3%) isolates; the β-lactamases genes (bla_{TEM} and bla_{NDM}) detected in 2 (13.3%) isolates and also 2(13.3%) isolates carried (bla_{TEM}) gene (figure 4.4, 4.5 and 4.6). Thus, the most isolates harbored more than one carbapenemase production genes. The sequencing analysis of bla_{OXA23}-like genes and bla_{OXA51}-like gene amplicons revealed that all isolates had an identical nucleotide sequence, but sequencing the bla_{TEM} and bla_{NDM} revealed nucleotide identify of *E. coli* and *Klebsiella pneumonia* plasmid genes. These new strains were named and submitted to the

GenBank under accession number OP572243 for *bla*$_{NDM}$ and OP572244 for *bla*$_{TEM}$. Screening results of the *β*-Lactamase genes in the bacterial plasmids, interestingly, all tested β-Lactamase genes were present in bacteria plasmids except *bla*$_{TEM}$. Furthermore, several genes which were absent in the isolate's chromosome existed on the isolate's plasmid. (table 4.5, appendix 1 and appendix 2).

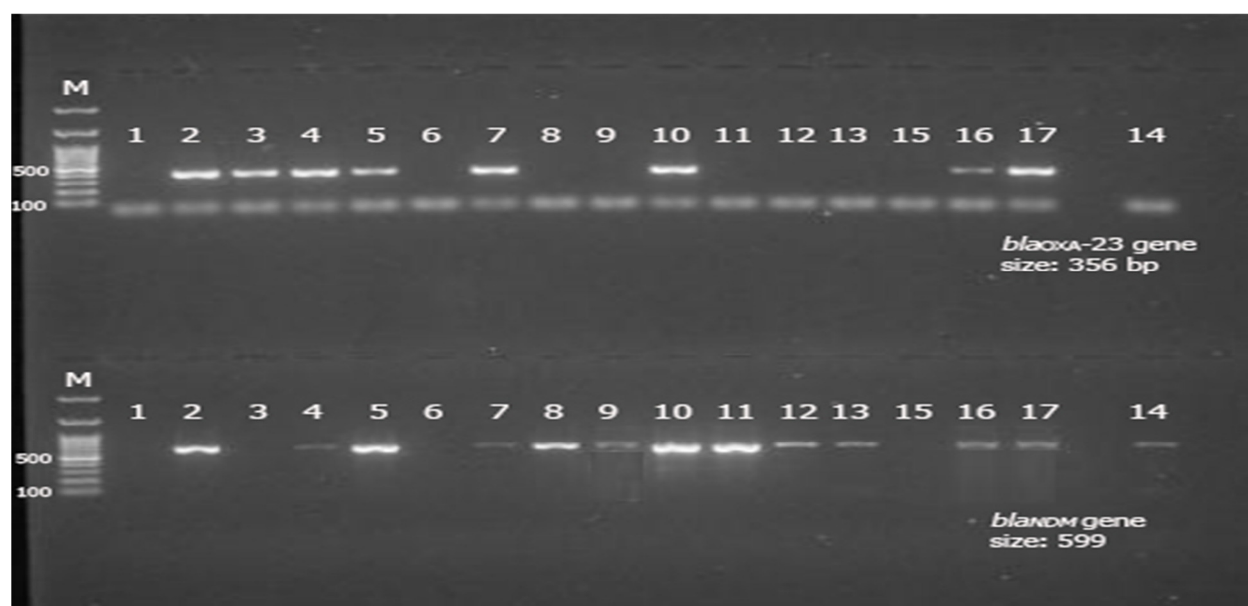

Figure 4.4. Gel electrophoresis of PCR products for resistance genes *bla*$_{OXA-23}$ (356 bp) in *A.baumannii*. Lane M is 100 bp DNA ladder; lane 2,3,4,5,7,10,16 and 17 show positive results for gene *bla*$_{OXA-23}$ (356 bp). And below one Gel electrophoresis for resistance genes *bla*$_{\mathrm{NDM}}$ (599 bp) in *A.baumannii* in which lane M is 100 bp DNA ladder, lane 2,4,5,7,8,9,10,11,12,13,14,16 and 17 show positive results for gene *bla*$_{OXA-23}$ (356 bp).

Figure 4.5. Gel electrophoresis of PCR products for resistance genes *bla*$_{\mathrm{TEM}}$ (720 bp) in *A. baumannii*. lane M is 100 bp DNA ladder; lane 1-17 show positive results for gene *bla*$_{\mathrm{TEM}}$ (720 bp).

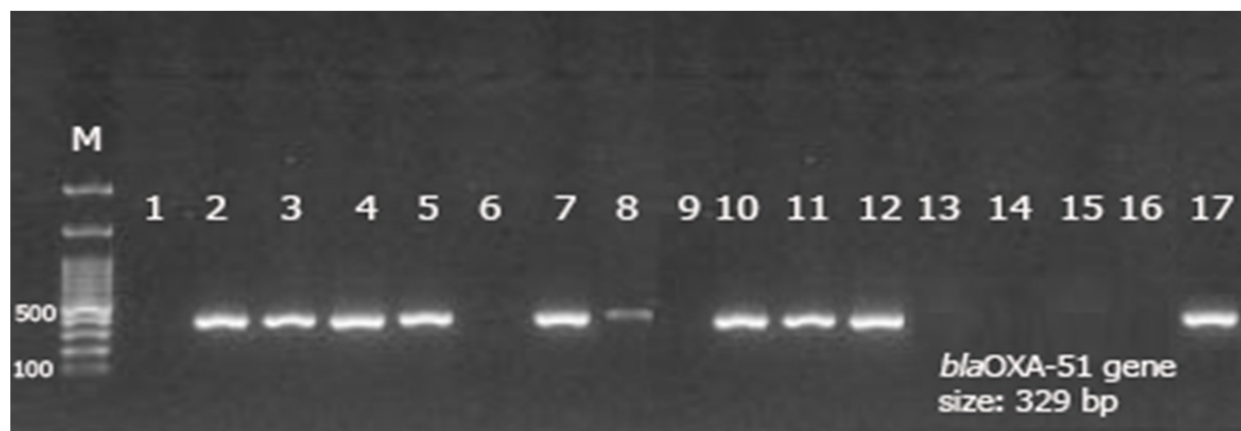

Figure 4.6. Gel electrophoresis of PCR products for resistance genes bla_{OXA-51} (329 bp) in *A. baumannii*. lane M is 100 bp DNA ladder, lane 2,3,4,5,7,8,10,11,12 and 17 show positive results for bla_{OXA-51} gene (329 bp).

4.2.7.2.2. Detection of antibiotics resistance genes

4.2.7.2.2.1. Gene encoding efflux pumps *adeB*

Efflux pump enhanced expression in *A. baumannii* is confer resistance to aminoglycosides, quinolones, tetracyclines, and trimethoprim. The efflux pump gene *adeB* is a part of AdeABC pump essential antibiotics resistance mechanism in *A. baumannii*. *adeB* gene was identified in most clinical isolates (Longo et al., 2014). This gene was found in 13 (76.5%) isolates chromosome, whilst all isolates plasmids harbored this gene (table 4.7, figure 4.7 and 4.8).

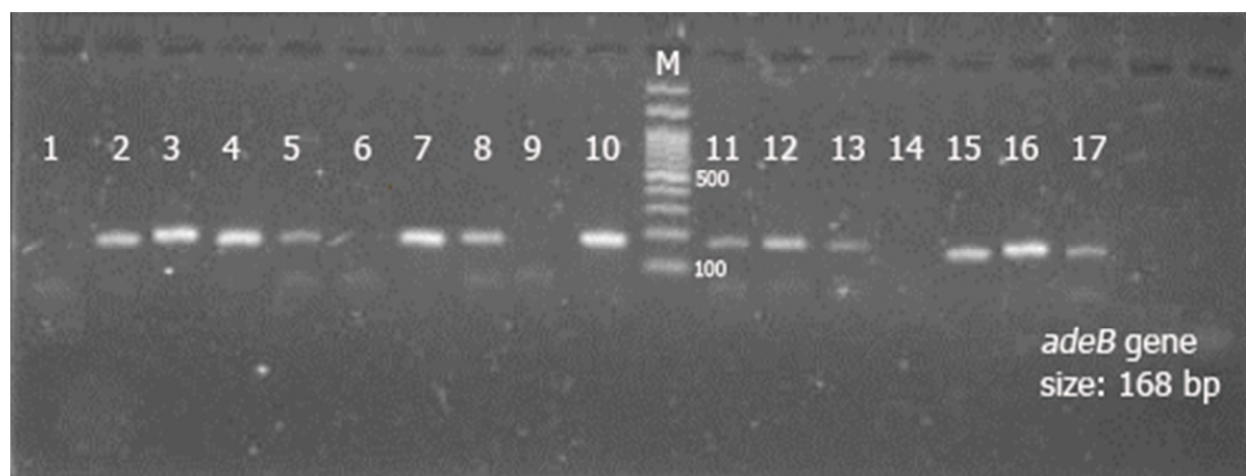

Figure 4.7. Gel electrophoresis of PCR products for resistance genes *adeB* (168 bp) in *A.baumannii*. Lane M is 100 bp DNA ladder, lane 2,3,4,5,7,8,10,11,12,13,15,16 and 17 show positive results and lane: 1,6,9 and 14 show negative results for gene *adeB* (168 bp).

Figure 4.8. Gel electrophoresis of PCR products for resistance genes *adeB* (168 bp) in *A. baumannii*. lane M is 100 bp DNA ladder, lane 1-17 show positive results for gene *adeB* in plasmids (168 bp).

4.2.7.2.2.2. Macrolides resistance encoding Gene *mph(E), msr(E), erm 42*

Lack of macrolides resistance genes were detected in chromosomal of *A. baumannii* isolates even in plasmids except for the *msrE* gene. The Msr protein responding resistance to macrolides (Huang et al., 2022). Whereas 5 (29.4%) isolated plasmids carried *msrE* gene (appendix 1, appendix 2, Table 4.7 and figure 4.9). The *erm(42)* gene encodes a monomethyltransferase that adds a single methyl group to 23S rRNA nucleotide A2058(*Escherichia coli* rRNA numbering system); however, *msr*(E) and *mph*(E) encode a macrolide efflux pump and a macrolide-inactivating phosphotransferase, respectively (Rose et al., 2012).

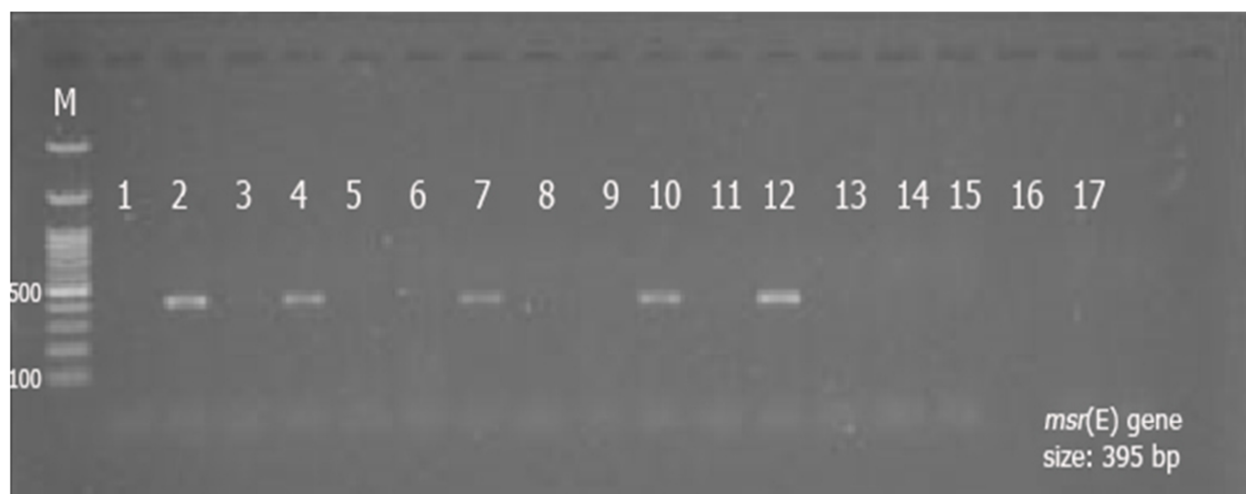

Figure 4.9. Gel electrophoresis for multiplex PCR products for resistance genes *msr(E)* (395 bp) in *A. baumannii*. Lane M is 100 bp DNA ladder, lane 2,4,7,10 and 12 show positive results for gene *msr(E)* (395 bp).

4.2.7.2.2.3. Aminoglycoside resistance encoding gene *aadB*, *aph(3')-VI* and *aacA4*

Aminoglycoside resistance gene *aadB* confers resistance to tobramycin, gentamicin, and kanamycin resistance. However, the 17 isolates chromosome and plasmid lacked the *aadB* gene. Meanwhile, the result showed that 29.4% and 64.7% isolates chromosome carried the aacA4 and *aph(3')-VI* genes respectively, whilst plasmid carried (88.2%) aacA4 and 100% *aph(3')-VI* resistance genes (figure 4.10 and 4.11). Three different functional groups of modifier enzymes are known including, aminoglycoside acetyltransferases (AAC), such as AAC (60)-Ih (which also confers resistance to gentamicin and amikacin), aminoglycoside phosphortrans-ferases (APH), such as APH (30)-IA (which confers resistance to gentamicin), and aminoglycoside adenylyltransferase (ANT), such as ANT (200)-IA (Vázquez-López et al., 2020). AG resistance genes can be transferred by means of mobilizable or conjugative plasmids, natural transformation, or transduction (Garneau & Labby, 2016).

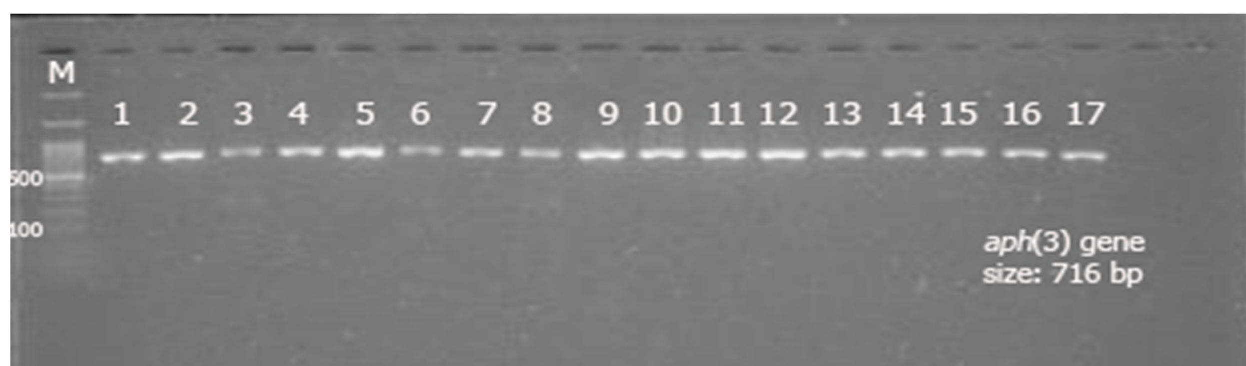

Figure 4.10. Gel electrophoresis of PCR products for resistance genes *aph(3')* (716 bp) in *A. baumannii.* lane M is 100 bp DNA ladder and lane 1-17 show positive results for genes *aph(3')* (716 bp).

Figure 4. 11. Gel electrophoresis of PCR products for resistance genes *aacA4* (450 bp) in *A. baumannii.* Land M is 100 bp DNA ladder and land 1,2,3,4,6,8,9,11,12,13,14,15,16 and 17 show positive results for gene *aacA4* (450 bp).

4.2.7.2.2.4. Streptomycin resistance encoding gene *strA* and *strB*.

The *strA* and *strB* are streptomycin resistant genes which coexisted in 11(64.7%) isolates chromosome DNA and inside all isolate's plasmid DNA (figure 4.12 and 4.13).

Figure 4.12. Gel electrophoresis of multiplex PCR products for resistance genes *strA* (165 bp) and *strB* (600bp) in *A. baumannii.* lane M is 100 bp DNA ladder and lane1,2,3,4,7,8,10,11,12,13 and 16 show positive results for *strA* (165 bp) and *strB* (600bp).

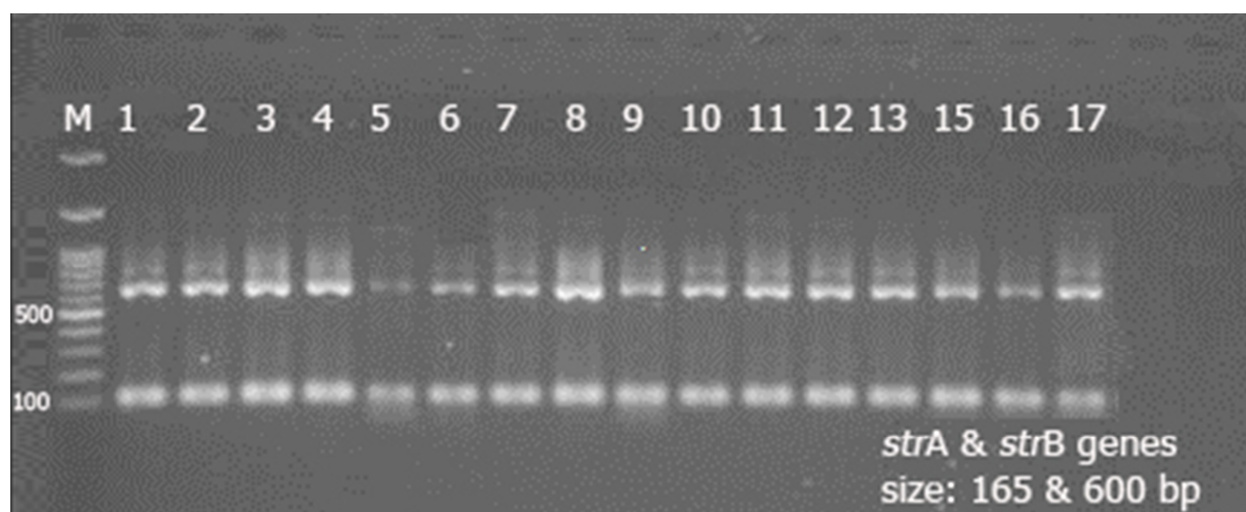

Figure 4.13. Gel electrophoresis of multiplex PCR products for resistance genes *strA* (165 bp) and *strB* (600bp) in *A. baumannii.* lane M is 100 bp DNA ladder and lane 1-17show positive results for *strA* (165 bp) and *strB* (600bp) .

4.2.7.2.2.5. Tetracycline resistance encoding gene *tetA* and *tetB*.

Of the 17 isolates analyzed by multiplex PCR, 1(5.9%) carry *tetA* gene, 11 (64.7%) carry *tetB* gene, while amplification of tetracycline resistance genes *tetA* and *tetB* in plasmids 4 (23.5%) and 13 (76.5%) respectively (figures 4.14).

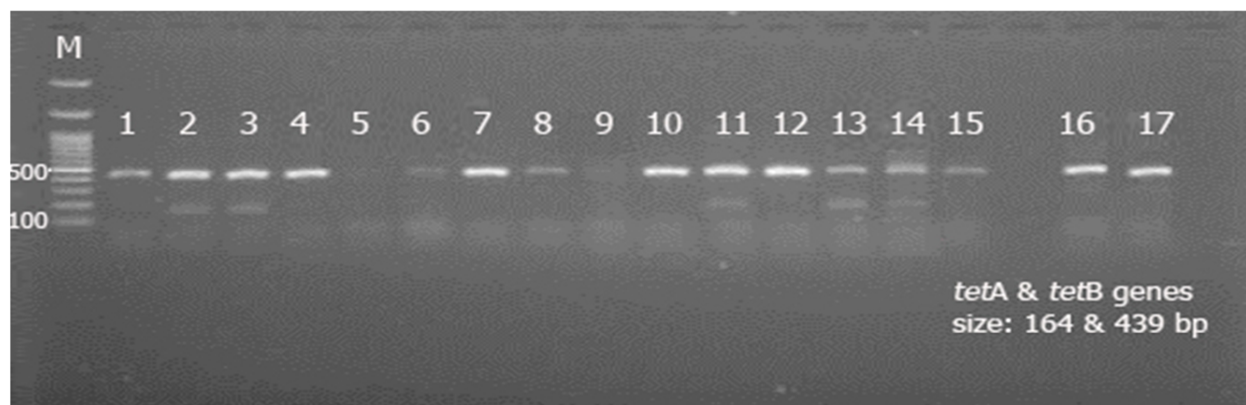

Figure 4.14. Gel electrophoresis of multiplex PCR products for resistance genes *tetA* (164 bp) and *tetB* (437 bp) in *A.baumannii.* Lane M is 100 bp DNA ladder and lane 1,2,3,4,6,7,8,10,11,12,13,14,15,16 and 17 shown the positive results for genes *tetB* (437 bp) and lane 2,3,11,13 and 14 negative results for *tetA (164 bp)* .

4.2.7.2.2.6. Fluoroquinolones resistance encoding gene *gyrA and parC*

The *parC* and *gyrA* genes amplification indicated the prevalence of the two genes in all strains which have been detected in all isolate's chromosome and plasmid (figures 4.15, 4.16, 4.17 and 4.18). Resistance to quinolones and fluoroquinolones can occur through mutations in the genes encoding the bacterial gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parA* and *parC*), that lower the binding affinity of these antibiotics to the enzyme-DNA complex (Poirel et al., 2011). The most commonly reported mutations that have been experimentally shown to confer resistance to fluoroquinolones such as ciprofloxacin are those that result in a Ser-81-Leu substitution in GyrA and a Ser-84-Leu substitution in ParC (Vázquez-López et al., 2020).

Figure 4.15. Gel electrophoresis of PCR products for resistance genes *gyrA* (350 bp) in *A. baumannii*. lane M is 100 bp DNA ladder and lane 1-17 show positive results for gene *gyrA* (350 bp) in plasmid.

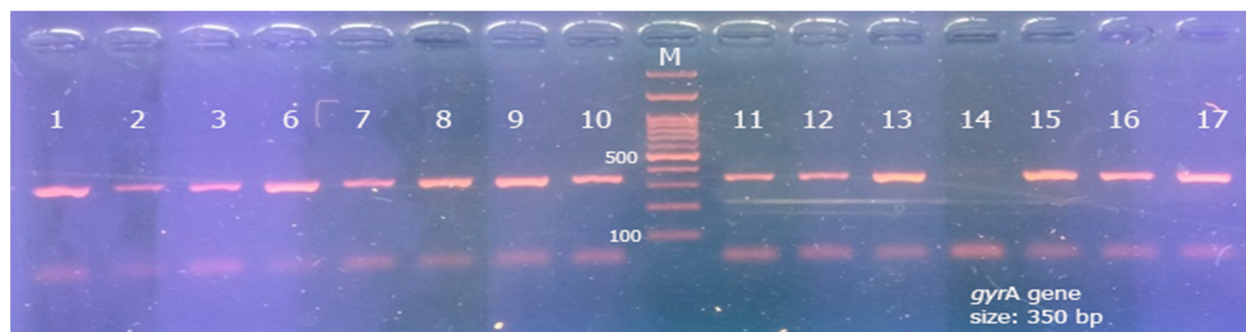

Figure 4.16. Gel electrophoresis of PCR products for resistance genes *gyrA* (350 bp) in *A. baumannii.* lane M is 100 bp DNA ladder and lane 1,2,3,6,7,8,9,10,11,12,13,15,16 and 17 show positive results for gene *gyrA* (350 bp) in chromosome.

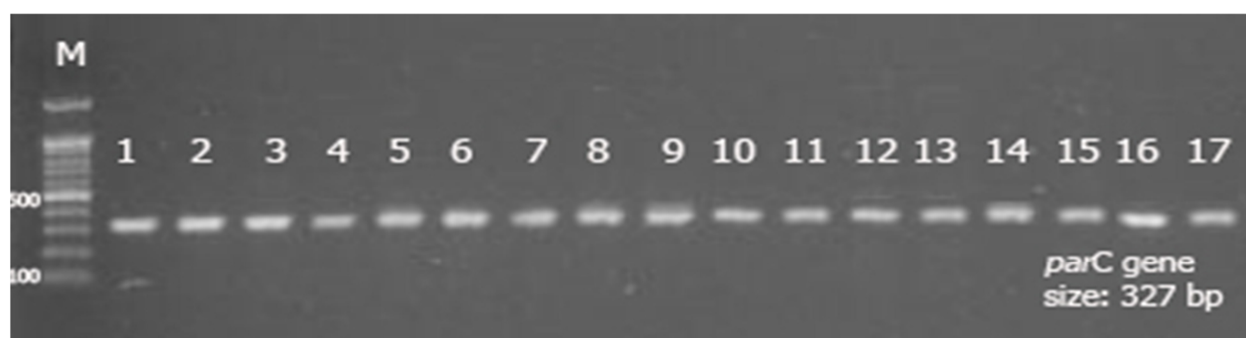

Figure 4.17. Gel electrophoresis of PCR products for resistance genes *parC* (327 bp) in *A. baumannii.* lane M is 100 bp DNA ladder and lane (1-17) show positive results for gene *parC* (327 bp) in chromosome.

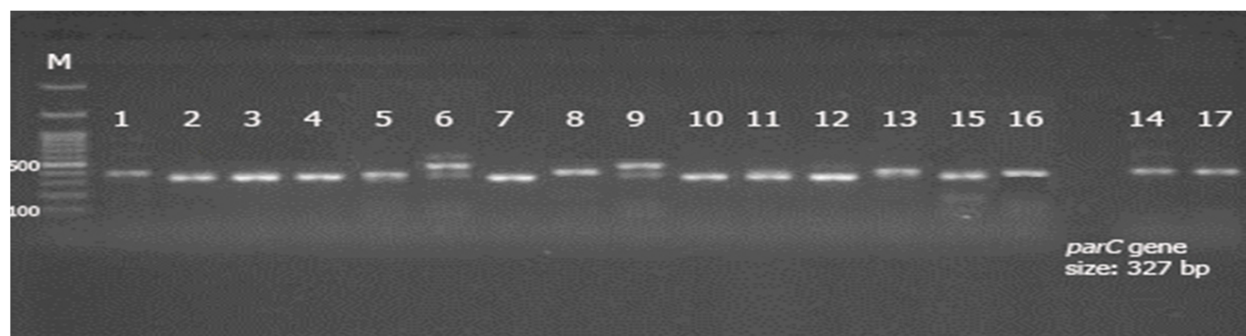

Figure 4.18. Gel electrophoresis of PCR products for resistance genes *parC* (327 bp) in *A. baumannii*. lanes M is 100 bp DNA ladder and lane (1-17) show having this positive results for gene *parC* (327 bp) in plasmid.

4.2.7.2.2.7. Sulfonamides resistance encoding gene *sul I, sul II*

The resistance sulfonamide genes were found in both chromosome and plasmid isolates. *Sul I* was detected in 11(64.7%) isolates, *Sul II* was encoded in 6 (35.3%) isolates (figure 4.19). Both genes were detected in all (100%) isolates' plasmid (figure 4.20). Sulphonamide resistance

is frequently detected in *A. baumannii* can also be conferred by *Sul I* and *Sul II* genes (Nigro & Hall 2012; Holt *et al.*, 2015).

Figure 4.19. Gel electrophoresis of multiplex PCR products for resistance genes *sul1* (433) and *sul2* (293) in *A. baumannii.* lane M is 100 bp DNA ladder and lane 2,3,4,6,7,9,10,11,12,13,14,15,16 17 show positive results for gene *sul1* (433), lane 1,6,8,9,11,13,15,16 and17 show positive results for gene *sul2* (293) in chromosome.

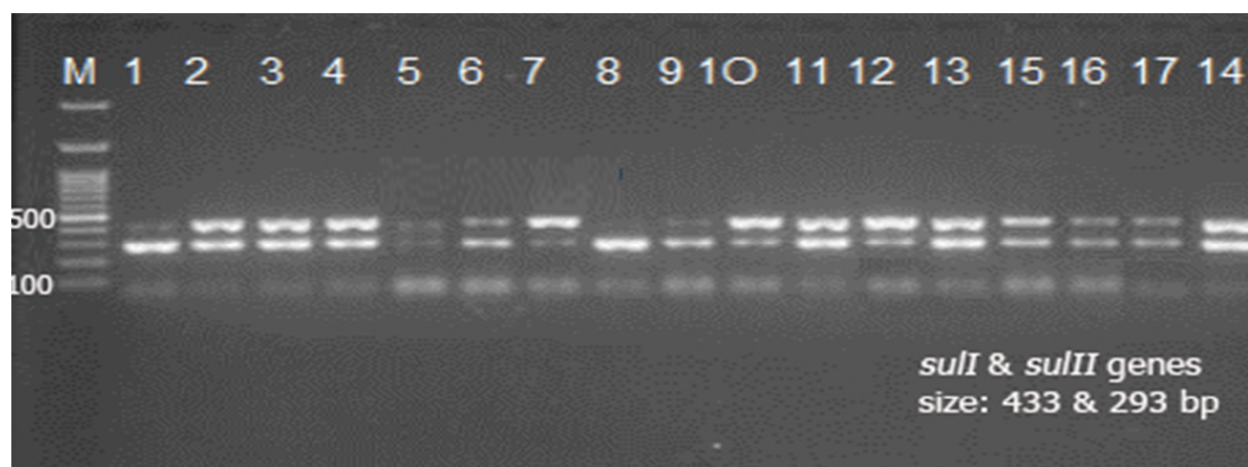

Figure 4.20: Gel electrophoresis of multiplex PCR products for resistance genes *sul1* (433) and *sul2* (293) in *A. baumannii*. lane M is 100 bp DNA ladder and lane 1-17 show positive results for both genes *sul1* (433) and *sul2* (293) in plasmid .

4.2.7.2.2.8. Trimethoprimresistance encoding gene *dhfr1*

Interestingly, trimethoprim gene *dhfr1* was encoded by only 5 (29.4%) plasmids isolated, whilst this gene was absent on isolates chromosome (figure 4.21). Resistance to trimethoprim can be associated with *dfr* genes located in gene cassettes in integrons or overexpression of intrinsic efflux pumps (Coyne et al., 2011).

Figure 4.21. Gel electrophoresis of PCR products for resistance genes *dhfr1* (220 bp) in *A. baumannii.* lanes M is 100 bp DNA ladder and lane 2,4,7,10 and 11 show positive results for gene *dhfr1* (220 bp) in plasmid.

Overall, the strains harbor several antibiotic genes that were acquired from other bacterial genera, though the macrolide and trimethoprim resistance genes were less detected genes of isolates (table 4.7).

Table 4.7. Percentage of antibiotics resistance genes detected in *A. baumannii* Isolates.

Antibiotic	Genes	% Detection in isolates chromosome DNA	% Detection in isolates plasmid DNA
Aminoglycoside	*StrA*	64.7	100
	StrB	64.7	100
	aadB	0	0
	aph(3')-VI	64.7	100
	aacA4	29.4	88.2
Multidrug efflux	*AdeB*	76.5	100
Sulfanamid	*Sul1*	64.7	100
	Sul2	40	100
Tetracycline	*TetA*	5.9	26.7
	TetB	64.7	76.5
Macrolide	*mph(E)*	0	0
	msr(E)	0	29.4
	erm(42)	0	0
Trimethoprime	*Dhfr1*	0	29.4
Fluoroquinolone	*gyrA*	100	100
	parC	100	100

Chapter Five

5. Discussion

The goal of the study was to determine the resistance characteristics in *A. baumannii*, taking a “snapshot” of this complex bacteria in case of epidemics. This study reveals that, the outbreak of multidrug resistance *A. baumannii* infections in hospitals accommodation is significant. The characteristic of the study is that Covid 19 patients (13%) were the most isolated clinical source of *A. baumannii.* This may revolve around the nature and severity of this disease among the patients: most of the patients were in ICU. Only the sources of seven (1.23%) isolates were urinary tract infection. Thus, the findings can be arranged into three stages, the first stage which was the isolate's characteristics and related to the pathogenesis, secondly antibiotics resistance, and emergence of epidemic disease, and finally, the sequencing of bacteria genes led to investigating alteration capacity of the isolates. The first feature in this study was biofilm formation, which was detected in 41.2% of isolates, despite the huge numbers of resistance genes found in all isolates; there is no strong biofilm formation, although many studies found that the *A. baumannii* multidrug resistance was associated with biofilm formation (Longo, Vuotto and Donelli, 2014; Saadati et al., 2021; Upmanyu, Haq and Singh, 2022); another study showed that 61.1% (33/54) of the isolates were strong biofilm producers, 27.7% (15/54) and 11.1% (6/54) showed moderate and weak biofilm production, respectively (Khalil et al., 2021). Likewise, Eze, El Zowalaty and Pillay (2021) revealed that 56.3%, 39.4%, and 4.3% were categorized as strong, moderate and weak biofilm-producing strains, respectively (Eze et al., 2021). Furthermore, in this study the plasmid was extracted in all isolates. In a bioinformatics study analysis, it was found that only 34.6% of *A. baumannii* plasmid carried multidrug resistance genes (Salgado et al., 2020). This result is compatible with other studies which found the plasmid in all extended-spectrum β-lactamases (ESBLs) positive- MDR-*A. baumannii* isolates carried a plasmid, and 90% ESBL-negative MDR- *A. baumannii* isolates, while no plasmid was found in 10% isolates (Al-Sheboul et al., 2022). Usually, *A. baumannii* small plasmid size is difficult to transfer, and thereby use alternative mechanisms for transformation. Moreover, the > 20 kb plasmid gene resistance are within mobile genetic elements such as transposons and integrons or excised phage. The large number of antibiotics resistance genes on plasmids contributed to promote survival of *A. baumannii* in clinical environment (Brovedan et al., 2020). A second striking character of this study is the huge number of antibiotic resistance genes determined in these

isolates. All isolates had eight or more resistance determinants. The genetic analysis revealed that the bla_{TEM} were found in all of the chromosome strains. More significantly, the study was also detected the presence of the bla_{NDM} in 76.5%, bla_{OXA}-51-like gene in 58.8 % and bla_{OXA}-23-like gene 29.4% with absent bla_{OXA}-58-like gene and bla_{SHV} on extracted chromosomal DNA.

In Nogbou *et al.* (2021); Al-Tamimi *et al.* (2022); and Ferjani *et al*. (2022) studies detected bla_{OXA-51} in 100% isolates. Kanaan and Khashan's (2022) study detected $bla_{OXA-51-like}$ in 76%, and Ghaffoori Kanaan *et al.* (2020) detected $bla_{OXA-51-like}$ in 66%, while bla_{OXA-23}-like gene was detected in 98.5% isolates (Al-Tamimi et al., 2022), and 100% in Ferjani *et al.*, (2022) study. On the other hand, Khalil *et al*'s (2021) study showned that the prevalence of carbapenemases- encoding genes among isolates, bla_{OXA-23}-like gene was positive in 88.9% of the isolates, the bla_{NDM} gene was found in 27.7% of the isolates (Khalil et al., 2021). Likewise, bla_{OXA-51} was detected (100%), bla_{OXA}-*23* (87.5%), bla_{OXA-24} (4.2%) and bla_{OXA-58} (0%) of the isolates (Al-Sheboul et al., 2022).

The bla_{NDM} was detected in other studies as 18.51% (Sales et al., 2021), and 20% of the strains (Mohammadi et al., 2020). A study in 2021 revealed prevalence of bla_{OXA-23} and bla_{OXA-51}, in 73% and 90% of the isolates, respectively, along with bla_{NDM} (92.2%) and bla_{OXA-58} gene was not detected in the isolates (Zahra et al., 2021). Another study detected 62% of bla_{OXA-23}-like gene and 22% of bla_{OXA-58} genes in isolates (Ghaffoori Kanaan et al., 2020). A previous study from Iraq reported that genotypically identified *A. baumannii* represented resistance to all of the investigated β–lactam antibiotics. Besides, bla_{OXA-51}, bla_{NDM}, and bla_{OXA-23} were seen in 100%, 62.5%, and 59.4% of isolates (Al-Kadmy et al., 2020). By contrast, the plasmid resistance genes bla_{TEM}, bla_{NDM}, bla_{OXA-51}-like gene, bla_{OXA}-*23*-like gene, bla_{OXA}-*58*-like gene and bla_{SHV} were detected in 0%, 47%, 17,64%, 41.2%, 17,64% and 23.5% respectively. Thus, the bla_{TEM} gene is dissemination in isolates chromosome and contribute to Piperacillin, Piperacillin/Tazobactam, and ceftazidime in 100%, cefepime, imipenem in 94% and meropenem resistance in 88,2 % of the carbapenem-resistant isolates in this collection.

This analysis contrasted to studies by Poirel, Naas and Nordmann, (2010); Yousefi Nojookambari *et al.* (2021), wherein intrinsic and chromosomally located *OXA-51-like* β-lactamases and acquired *OXA-23-like*, *OXA-58-like* β-lactamases was found in carbapenem-resistant *A. baumannii* isolates, bla_{OXA-23} was found to be responsible for imipenem resistance in

the majority of strains and raises resistance mechanisms by horizontal gene transfer (Huang et al., 2012). In addition to the most common carbapenem-resistant *A. baumannii* mechanisms is presence of oxacillinases *OXA-23, OXA-58* and *NDM-like* β-lactamases (Nguyen & Joshi, 2021), carbapenem-resistant *A. baumannii* class D in this study belongs to international clonal lineage II (ICL-II), found mostly in ICUs (Minandri et al., 2012).

The detection of new nucleotide sequences of beta lactamase genes was identified in this study in different isolates (table 4.6 and Appendices 5-9). These genes sequences, located in the bla_{NDM} and bla_{TEM} genes were acquired from the *Enterobacteriaceae* genus by transferring mobile genetics elements in the ICU patients that were identified as coinfections and might act as a potent link to higher levels of carbapenem resistance except for colistin (figure 5.1, 5.2, Appendix 3 and 4). The other B-lactamase gene sequences analysed in this study identified in the NCBI gene bank after *Covid19* outbreaks, this may be an alarm to promoting revolution epidemic *A. baumannii* resistance genes in any disease outbreaks. The sequence analysis has also shown the new strain *Stenotrophomones maltophilia* strain HK (accession number in gene bank OP422244.1) with to new genes including, *A. baumannii* B-lactamase, bla_{oxa-23} genes (accession number in gene bank OP595162.1) and bla_{NDM} genes (accession number in gene bank OP595163.1) (Appendices 5-7).

Acinetobacter baumannii strain carbapenems resistance HK19 (accession number: OP572243)

Escherichia coli strain 779 plasmid p779-5-NDM, complete sequence

Escherichia coli strain JH51 plasmid pJH51-1, complete sequence

Escherichia coli strain b75c plasmid p_b75c_NDM5, complete sequence

Klebsiella pneumoniae strain dm651b plasmid p_dm651b_NDM5, complete sequence

Klebsiella pneumoniae strain b156b plasmid p1_b156b_NDM5, complete sequence

Klebsiella pneumoniae strain dm664b plasmid p2_dm664b_NDM5, complete sequence

Klebsiella pneumoniae strain b119 plasmid p_b119_NDM5, complete sequence

Escherichia coli strain b148b plasmid p_b148b_NDM5, complete sequence

Klebsiella pneumoniae strain b199b plasmid p_b199b_NDM5, complete sequence

Figure 5.1. distance tree of the new *blaNDM* gene of *A. baumannii* (accession number in gene bank OP572243) which was isolated from Covid19 ICU patients demonstrating the origin of the new gene from horizontal gene transfer.

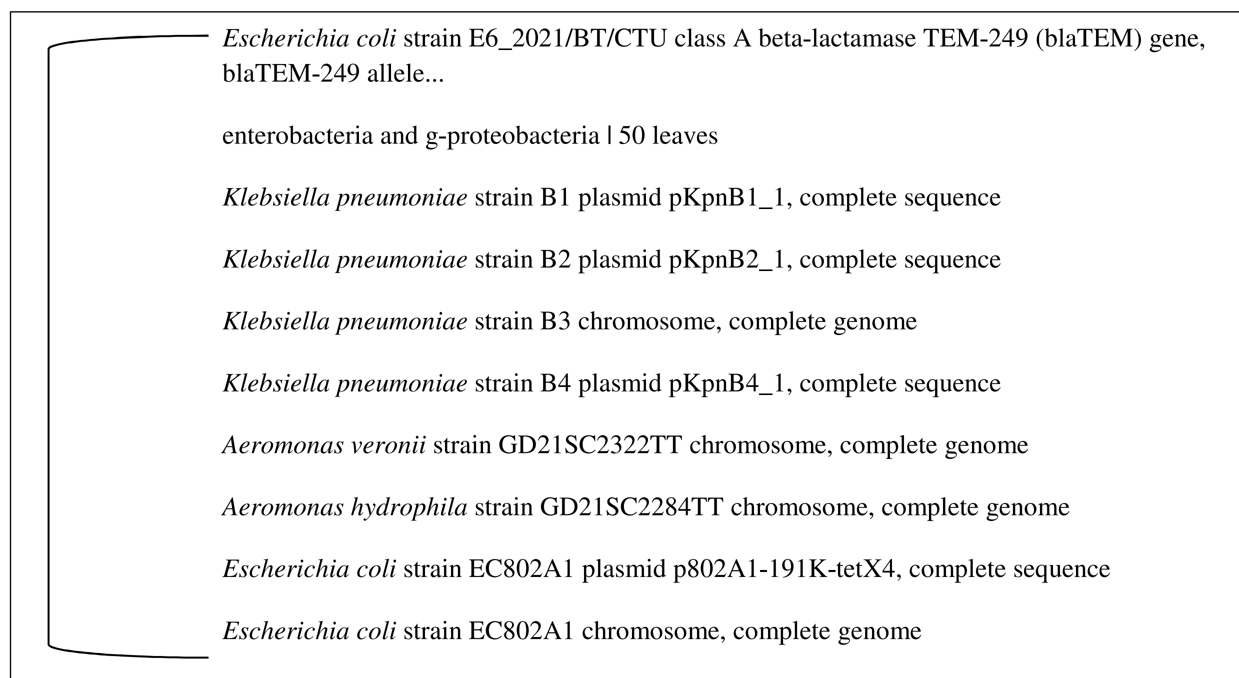

Figure 5.2. Distance tree of the new *blaTEM* gene of *A. baumannii* (accession number in gene bank OP572244) which was isolated from Covid19 ICU patients demonstrating the origin of the new gene from horizontal gene transfer.

Most importantly, carbapenemase producing *Acinetobacter spp*. were disseminated in many Erbil hospitals, according to the current study, nearly 100% of isolates from these hospitals, produced beta-lactamase. Our present study also noted that Extended-spectrum β-lactamases (ESBLs) producing isolates including bla_{TEM} and bla_{SHV} were much more prevalent (100%) than those with OXA β-lactamases producing. bla_{TEM} and bla_{SHV} confer resistance to cephalosporins (cefotaxime, ceftriaxone, ceftazidime, and aztreonam). It is interesting that the new bla_{TEM} carrying isolates were detected (table 4.5). Further, bla_{TEM} and bla_{SHV} encoded frequently by plasmids carry resistance to other antibiotics classes (Paterson & Bonomo, 2005), making clear the high numbers of different resistance genes in isolated plasmids in this study. The bla_{TEM} encoded 100% by chromosome whereas bla_{SHV} encoded 26.7% by plasmid. Consequently, the role of plasmid is essential in gene transfer to bacteria chromosomes to increase *A. baumannii* pathogenicity.

It is obvious that isolates that carry resistance genes *gyrA* and *parC* to fluoroquinolone class on both plasmids and chromosomes confers phenotypically resistance between 94% to ciprofloxacin and 100% to levofloxacin. An Egyptian study found that 90% of the isolates had *gyrA* and *parC* mutations (Ser 81 → Leu mutation for *gyr*A gene and Ser 84 → Leu mutation for *par*C gene) (Mohammed et al., 2021). Moreover, a South Africa study has detected *parC* chromosomal gene mutation in 60% of isolates and *gyrA* chromosomal gene mutation in 85% of isolates (Nogbou et al., 2021). *A. baumannii* is mainly resistant to quinolones through chromosomal gene mutation in *parC* and *gyrA* (Singh *et al.*, 2020; Nogbou *et al.*, 2021). However, in this study *parC* and *gyrA* genes mutation detected in 100% of isolates both in plasmids and chromosomes. The resistance to gentamycin, netilmicin and tobramycin was 64.7%, 53.8% and 57% respectively. Interestingly the resistance genes of these groups encode *strAB* and *aph(3')-VI* 100% in plasmids and 73.3% in chromosomes except *aadB* which was not encoded by bacteria, *aadB* gene encodes to tobramycin resistance(Hujer et al., 2006b). The *strAB* in our study have more prevalence compared with Egyptian study which was found in 44.4% of isolates (Hamed et al., 2022). Efflux pump genes *aacA4* which related to aminoglycosides resistance encoded 88.2% in plasmid and 29.4% in chromosome, whereas the *AdeB* gene was involved in the resistance to other antibiotics such as tigecycline, beta-lactams, chloramphenicol, erythromycin, and tetracycline as well encodes 100% in plasmid and 86.7% in chromosome. ELsheredy *et al*'s (2021) study in Egypt detected *aacA4* gene and *aadB* gene in 37% and 4% of the isolates (ELsheredy et al., 2021). Tetracycline resistance was 92% while the genes encoded 5.9% *TetA*, and 64.7% *TetB* on chromosome and 23.5% *TetA,* and 76.5% *TetB* on plasmid. Consistent with this study, Kanaan and Khashan's (2022) study in Baghdad/Iraq detected *TetA* in 98% of isolates and 96% of *TetB*. Further, a study reported 80% of *TetA* and 70.5% of *TetB* genes in the isolates (Ghaffoori Kanaan et al., 2020). By contrast, an Iranian study showed that 32.40% *TetA* gene and 21.29% of *TetB* gene detected in the isolates (Sales et al., 2021). Besides Meshkat et al's 2021 study in Iran showed that prevalence of tetracycline resistance genes were 86.7% and 3.33% *TetB* and *TetA* (Meshkat et al., 2021).

The resistance genes for both macrolide including (*erm (42), msr(E) and mph(E))* and trimethoprim (*Dhfr1)* were not detected in chromosomes of isolates; however, *msr(E)* and *Dhfr1* genes were the least encoded genes at 29.4% on plasmids, therefore these antibiotics were

effective for the majority of isolates. It should be noted that only 13% of *msr-I* gene resistance in isolates was detected by Kannan et al. (2020).

The combination of trimethoprim with sulfonamide resistance was 82.4%. This might be due to the presence of the resistance genes (*Sul1* and *Sul2* in 100% plasmids with 64.7% *Sul1* and 35.5% *Sul2* in chromosome). This study is in agreement with Kanaan and Khashan's (2022) study in Baghdad/Iraq which detected *Sul1* gene in 75% of isolates (Kanaan & Khashan, 2022), similarly, Hussain *et al*, reported 89.47% of *Sul1* gene resistance in isolates (E. A. Hussain et al., 2022). In contrast, 43% of *Sul1*gene resistance in isolates was detected in a study by Kanaan et al's 2020. Another study in Pakistan reported that 16.6% *sul1* and 20% *sul2* were detected in isolates (Zahra et al., 2021).

Apart from the role of metabolic pathways that related to bacteria survival and pathogenicity (appendix 12), the study showed that 93.8% of isolates metabolize D-cellobiose, D– glucose, D–mannose, malonate, L–lactate alkalinication, succinate alkalinication, and 100% coumarate. These metabolic pathways were involved in bacteria virulence determinants (Wu et al., 2012). Interestingly, 87.5% of isolates utilized of the sodium citrate and Glutamyl Arylamidace pNA. Arylamidase activities specific for basic amino acid hydrolysis, host tyrosine hydrolysis by specific bacterial virulence factor (Fukasawa *et al.*, 1982; Bliska *et al.*, 1991). Moreover, sodium citrate and malonate inhibited bacterial biofilm formation and decrease virulence factors (Khayat *et al.*, 2022; Elmassry *et al.*, 2021).

On the other hand, utilization of L–Proline arylamidase was 18.8%, Tyrocine arylamidase were 81.3%, urease was 43.8%, L–histidine accimilation and L–malate accimilation were 37.5 %, Ellman was 12.5 %, and L–lactate accimilation was 56.3%. *A. baumannii* reduced catabolism of mannitol and glutamate to survive in the dry hospital environment, this shifting in metabolism pathway, decreases growth, and unable to metabolize various amino acids by gene mutation (König et al., 2021). Metabolic role of the isolates to invade the immune system and causing inflammation with sepsis through phenylacetic acid catabolism pathway using coumarate enzyme 100% in all isolates, the coumarate (coenzyme A ligase) role is evading host immune (Kaneko, Ohnishi and Horinouchi, 2003; Bhuiyan *et al.*, 2016). Whenever the neutrophile activated against the pathogen bacteria produce reactive nitrogen species, and the pathogens to

survive and increase risk of invasion shift many metabolic pathways (Spahich *et al.*, 2016; Richardson *et al.*, 2011).

The wholistic analysis carried out in this study is the first study to link the multidrug resistance phenotypes and genetic determinants of resistance to β-lactams, aminoglycosides, fluoroquinolone, tetracycline, sulfonamide, trimethoprim, and macrolides in *A. baumannii* in the Erbil/ Iraq. Many of these *A. baumannii* isolates save as reservoirs for antibiotic resistance genes that were transmitted to other pathogens by plasmid. Besides the prevalent bla_{TEM}, a new bla_{TEM} and bla_{NDM} have emerged. Additionally, metabolism pathways can be an attractive therapeutic approach as this study clarifies the strain's metabolic pathway roles for the isolate’s characterizations and resistance determinants. Fermentation pathway increases gene transfer and integration of bacteria (Philipps, de Vries and Jennewein, 2019; San Millan *et al.*, 2018). The diversity of the new finding’s resistance determinants and the potential for broad distribution of these strains could alter the worldwide epidemiology of *A. baumannii*-related diseases.

Chapter Six

6. Conclusions and Recommendations

6.1. Conclusions

The conclusions of this study could be outlined as follows:

1. *A. baumannii* identification should be based on more than one primer.
2. All isolates which were identified as *A. baumannii* carried plasmid.
3. All isolates chromosome harbored the genes bla_{TEM}, *parC,* and *gyrA*, while *parC, gyrA, aph(3)VI, AdeB, sul I, sul II, strA,* and *strB* were harbored in isolates plasmid.
4. The isolates used the coumarate metabolic pathway and carbohydrate metabolic pathway more than the amino acid metabolic pathway.
5. The gene bla_{OXA-51} was not the ubiquitous gene in *A. baumannii* and was carried by several isolates' plasmid.
6. Sputum and urine specimens of patients were the sources of *A. baumannii* isolation.
7. COVID-19 sputum specimens were mostly *A. baumannii* isolates.
8. Resistance genes on the isolate's plasmid were encoded more than the isolates chromosome.
9. There was no strong biofilm formation.
10. All strains survive through the coumarate metabolic pathway.
11. All isolates were sensitive to colistin antibiotic phenotypically.
12. Trimethoprim and macrolides are the best choices for *A. baumannii* infection treatment.
13. bla_{TEM} was the disseminated gene in isolates and it was the unique gene that was absent on plasmid.

6.2. Recommendations

This opportunistic bacterium has proved by this study that is dangerous bacteria in epidemic or pandemic disease; thus, we recommend the followings:

1. Expanding the resistance genes classes study.
2. The plasmid study should be expanded including metabolism genes detection and sequencing.
3. *A. baumannii* identification methods should be studied more extensively.
4. bla_{TEM} gene requires more investigation in Kurdistan region.
5. The isolates that lack $bla_{oxa\text{-}51}$ requires depth study phenotypically and genetically.

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Appendices:

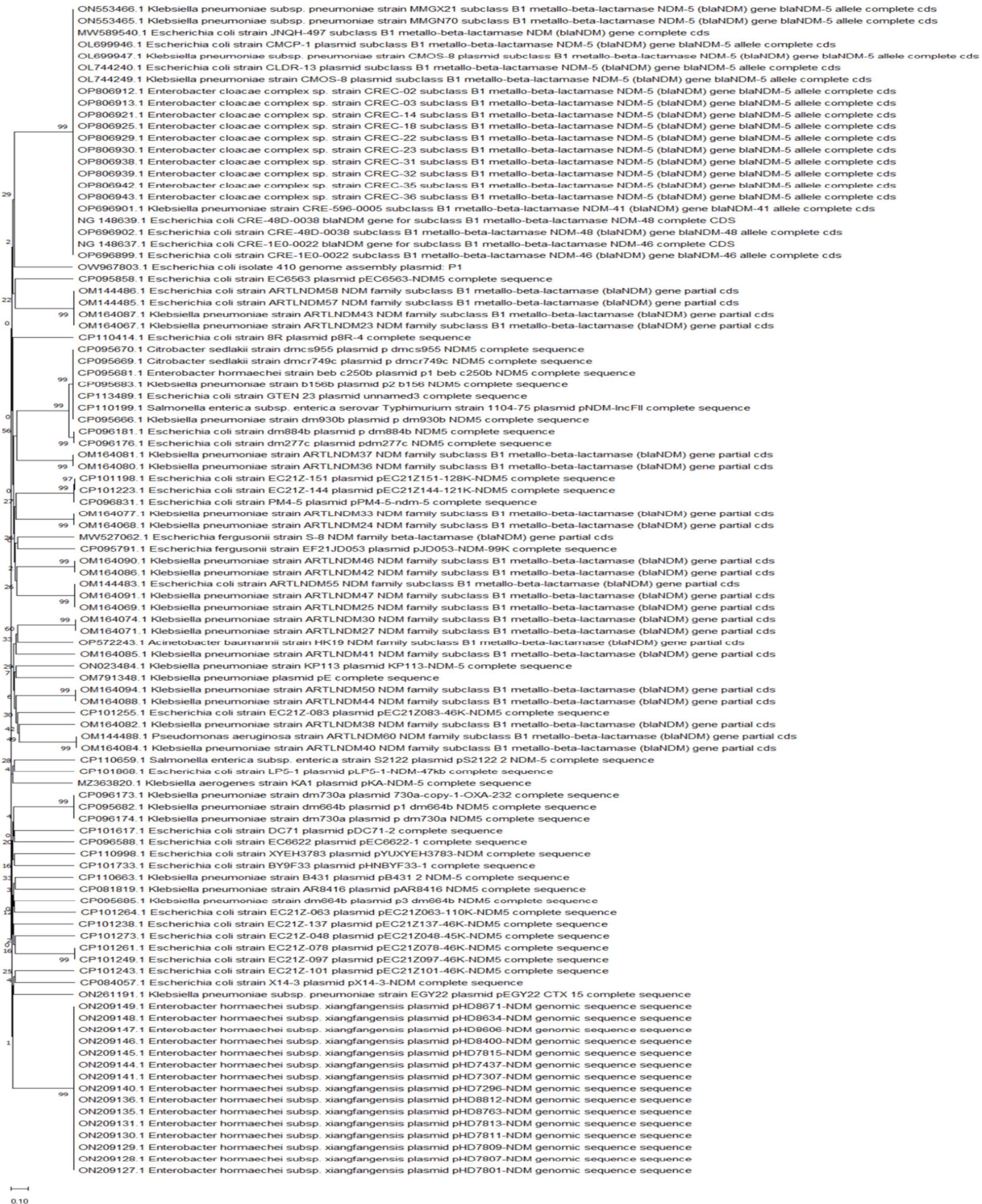
Appendix 1: *A*. *baumannii* isolates chromosome resistance genes.

Isolate	parC	gyrA	aadB	aacA4	aph(3) IV	AdeB	sul 1	sul 2	strA	strB	erm	mph	msr	tetA	tetB	dhfr1	Total genes no.
1	+	+	-	-	+	-	-	+	+	+	-	-	-	-	+	-	7
2	+	+	-	-	+	+	+	-	+	+	-	-	-	-	+	-	8
3	+	+	-	-	+	+	+	-	+	+	-	-	-	-	+	-	8
4	+	+	-	-	+	+	+	-	+	+	-	-	-	-	+	-	8
5	+	+	-	-	+	+	-	-	-	-	-	-	-	-	+	-	5
6	+	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-	5
7	+	+	-	-	+	+	+	-	+	+	-	-	-	-	+	-	8
8	+	+	-	-	+	+	-	+	+	+	-	-	-	-	+	-	8
9	+	+	-	+	+	-	+	+	-	-	-	-	-	-	-	-	6
10	+	+	-	+	+	+	+	-	+	+	-	-	-	-	+	-	9
11	+	+	-	-	+	+	-	-	+	+	-	-	-	-	+	-	7
12	+	+	-	-	-	+	+	-	+	+	-	-	-	-	+	-	7
13	+	+	-	+	-	+	+	+	+	+	-	-	-	+	+	-	10
14	+	Nil	-	+	-	-	+	-	-	-	-	-	Nil	-	+	+	5
15	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	5
16	+	+	-	+	-	+	+	-	+	+	-	-	-	-	-	-	7
17	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	5
Total %	100	100	0	26.7	73.3	86.7	73.3	40	73.3	73.3	0	0	0	6.7	73.3	0	

Appendix 2: *A. baumannii* isolates plasmid resistance genes.

isolate	parC	gyrA	aadB	aacA4	aph(3)VI	AdeB	sul1	sul2	strA	strB	erm	mph	msr	tet A	tet B	dhfr1	Total genes no.
1	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+	-	10
2	+	+	-	+	+	+	+	+	+	+	-	-	+	+	+	+	13
3	+	+	-	+	+	+	+	+	+	+	-	-	-	+	+	-	11
4	+	+	-	+	+	+	+	+	+	+	-	-	+	-	+	+	12
5	+	+	-	-	+	+	+	+	+	+	-	-	-	-	-	-	8
6	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+	-	10
7	+	+	-	-	+	+	+	+	+	+	-	-	+	-	+	+	11
8	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+	-	10
9	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+	-	10
10	+	+	-	+	+	+	+	+	+	+	-	-	+	-	+	+	12
11	+	+	-	+	+	+	+	+	+	+	-	-	-	+	+	+	12
12	+	+	-	+	+	+	+	+	+	+	-	-	+	-	+	-	11
13	+	+	-	+	+	+	+	+	+	+	-	-	-	+	+	-	11
14	+	+	-	+	+	+	+	+	+	+	-	-	-	+	+	-	11
15	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+	-	10
16	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+	-	10
17*	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+	-	8
Total %	100	100	0	86.7	100	100	100	100	100	100	0	0	33.3	26.7	93.3	33.3	

Appendix 3: distance tree of the new *bla_{NDM}* gene of *A. baumannii* (accession number in gene bank OP572243) which was isolated from Covid19 ICU patients demonstrating the origin of the new gene from horizontal gene transfer.



Appendix 4: distance tree of the new blaTEM gene of A. baumannii (accession number in gene bank OP572244) which was isolated from Covid19 ICU patients demonstrating the origin of the new gene from horizontal gene transfer.

OL629040.1:52-753 Escherichia coli strain GME4 TEM family beta-lactamase (blaTEM) gene partial cds
ON777848.1:15682-16383 Klebsiella pneumoniae subsp. pneumoniae strain 15WZ-61 plasmid p15WZ61-KPC
OL629041.1:55-756 Escherichia coli strain BME2 TEM family beta-lactamase (blaTEM) gene partial cds
OL629042.1:49-750 Escherichia coli strain GHE16 TEM family beta-lactamase (blaTEM) gene partial cds
OL629043.1:73-774 Escherichia coli strain GSE17 TEM family beta-lactamase (blaTEM) gene partial cds
CP104315.1:116511-117212 Klebsiella pneumoniae strain KP8132 plasmid pKP81323 complete sequence
CP104327.1:37973-38674 Escherichia coli strain THB42-F1 plasmid pHB42-F1 complete sequence
CP104331.1:99703-100404 Escherichia coli strain THB42-F3 plasmid pHB42-F3 complete sequence
CP104342.1:69649-70350 Acinetobacter baumannii strain 2021CK-01300 chromosome complete genome
CP104351.1:69649-70350 Acinetobacter baumannii strain 2021CK-01335 chromosome complete genome
CP104340.1:2921719-2922420 Acinetobacter baumannii strain 2021CK-01409 chromosome complete genome
CP104347.1:3803396-3804097 Acinetobacter baumannii strain 2021CK-01332 chromosome complete genome
CP031846.1:3930121-3930822 Proteus mirabilis strain XH983 chromosome complete genome
CP104489.1:17106-17807 Salmonella enterica strain SalSpp sample 10 No.2 plasmid unnamed1 complete sequence
CP104485.1:26293-26994 Salmonella enterica strain SalSpp sample 07 No.3 plasmid unnamed1 complete sequence
CP104480.1:17106-17807 Salmonella enterica strain SalSpp sample 09 No.5 plasmid unnamed1 complete sequence
CP100077.1:122802-123503 Klebsiella pneumoniae strain RJKP36 plasmid pRJKP36-1 complete sequence
CP100085.1:94000-94701 Klebsiella pneumoniae strain HSKP86 plasmid pHSKP86-1 complete sequence
CP100089.1:168462-169163 Klebsiella pneumoniae strain HSKP8 plasmid pHSKP8-2 complete sequence
CP100095.1:94000-94701 Klebsiella pneumoniae strain HSKP5 plasmid pHSKP5-1 complete sequence
CP100099.1:94000-94701 Klebsiella pneumoniae strain HSKP43 plasmid pHSKP43-1 complete sequence
CP100107.1:94000-94701 Klebsiella pneumoniae strain HSKP107 plasmid pHSKP107-1 complete sequence
CP100111.1:94000-94701 Klebsiella pneumoniae strain HSKP104 plasmid pHSKP104-1 complete sequence
CP100120.1:669695-670396 Klebsiella pneumoniae strain GZKP13 chromosome complete genome
CP100121.1:98263-98964 Klebsiella pneumoniae strain GZKP13 plasmid pGZKP13-1 complete sequence
CP100121.1:73360-74061 Klebsiella pneumoniae strain GZKP13 plasmid pGZKP13-1 complete sequence
AP026963.1:51820-52521 Escherichia coli EC20-C72-1 plasmid pEC20-C72-1-2 DNA complete sequence
CP109601.1:2701735-2702436 Escherichia coli strain TL-14 chromosome complete genome
CP109772.1:677298-677999 Klebsiella pneumoniae strain YZ-58 chromosome complete genome
CP109950.1:18629-19330 Klebsiella pneumoniae strain KP1650 plasmid pKP1650-2 complete sequence
CP109975.1:13587-14288 Klebsiella pneumoniae strain 857 plasmid pB complete sequence
CP110199.1:16074-16775 Salmonella enterica subsp. enterica serovar Typhimurium strain 1104-75 plasmid pNDM-IncFII complete sequence
CP110413.1:1137-1838 Escherichia coli strain 8R plasmid p8R-3 complete sequence
CP102701.1:106358-107059 Escherichia coli strain 9A-1-1 plasmid pE2899 complete sequence
CP102707.1:50525-51226 Escherichia coli strain 9A-3-9 plasmid pE2899 complete sequence
OP572244.1:1-702 Acinetobacter baumannii strain HK22 TEM family class A beta-lactamase (blaTEM) gene partial cds
OL744243.1:103-804 Escherichia coli strain CMW-3 plasmid broad-spectrum class A beta-lactamase TEM-1 (blaTEM) gene blaTEM-1 allele complete cds
OL744252.1:103-804 Klebsiella pneumoniae strain CBDY-10 plasmid broad-spectrum class A beta-lactamase TEM-1 (blaTEM) gene blaTEM-1 allele complete cds
CP110657.1:928209-928910 Salmonella enterica subsp. enterica strain S2122 chromosome complete genome
CP094368.3:164403-165104 Klebsiella pneumoniae strain SCPM-O-B-8922 (20PKP/19c) plasmid pB-8922 OXA-48 complete sequence
CP113541.1:4244237-4244938 Salmonella enterica strain CHC chromosome complete genome
CP113535.1:1242746-1243447 Salmonella enterica strain ZLQ chromosome complete genome
CP113792.1:7583-8284 Klebsiella variicola subsp. tropica strain BSK 177V2 plasmid pBSKP3 complete sequence
ON934549.1:42111-42812 Escherichia coli plasmid p13Q15 complete sequence
CP091608.1:54671-55372 Salmonella enterica strain 1810 plasmid p1810-MDR complete sequence
CP091616.1:64726-65427 Salmonella enterica strain 1653 plasmid p1653-MDR complete sequence
CP091627.1:64726-65427 Salmonella enterica strain 1165 plasmid p1165-MDR complete sequence
CP091631.1:64726-65427 Salmonella enterica strain 1147 plasmid p1147-MDR complete sequence
CP091637.1:130586-131287 Salmonella enterica strain 1063 plasmid p1063-MDR complete sequence
OP806952.1:103-804 Enterobacter cloacae complex sp. strain CREC-01 TEM family class A beta-lactamase (blaTEM) gene partial cds
OP806953.1:103-804 Enterobacter cloacae complex sp. strain CREC-04 TEM family class A beta-lactamase (blaTEM) gene partial cds
OP806954.1:103-804 Enterobacter cloacae complex sp. strain CREC-05 TEM family class A beta-lactamase (blaTEM) gene partial cds
OP806955.1:103-804 Enterobacter cloacae complex sp. strain CREC-06 TEM family class A beta-lactamase (blaTEM) gene partial cds
OP806956.1:103-804 Enterobacter cloacae complex sp. strain CREC-07 TEM family class A beta-lactamase (blaTEM) gene partial cds
OP806957.1:103-804 Enterobacter cloacae complex sp. strain CREC-08 TEM family class A beta-lactamase (blaTEM) gene partial cds
OP806958.1:103-804 Enterobacter cloacae complex sp. strain CREC-10 TEM family class A beta-lactamase (blaTEM) gene partial cds
OP806959.1:103-804 Enterobacter cloacae complex sp. strain CREC-13 TEM family class A beta-lactamase (blaTEM) gene partial cds
OP806960.1:103-804 Enterobacter cloacae complex sp. strain CREC-15 TEM family class A beta-lactamase (blaTEM) gene partial cds
OP806961.1:103-804 Enterobacter cloacae complex sp. strain CREC-16 TEM family class A beta-lactamase (blaTEM) gene partial cds
OP806962.1:103-804 Enterobacter cloacae complex sp. strain CREC-17 TEM family class A beta-lactamase (blaTEM) gene partial cds
OP806963.1:103-804 Enterobacter cloacae complex sp. strain CREC-19 TEM family class A beta-lactamase (blaTEM) gene partial cds
OP806964.1:103-804 Enterobacter cloacae complex sp. strain CREC-24 TEM family class A beta-lactamase (blaTEM) gene partial cds
OP806965.1:103-804 Enterobacter cloacae complex sp. strain CREC-25 TEM family class A beta-lactamase (blaTEM) gene partial cds
OP806966.1:103-804 Enterobacter cloacae complex sp. strain CREC-26 TEM family class A beta-lactamase (blaTEM) gene partial cds
OP806967.1:103-804 Enterobacter cloacae complex sp. strain CREC-27 TEM family class A beta-lactamase (blaTEM) gene partial cds
OP806968.1:103-804 Enterobacter cloacae complex sp. strain CREC-28 TEM family class A beta-lactamase (blaTEM) gene partial cds
OP806970.1:103-804 Enterobacter cloacae complex sp. strain CREC-33 TEM family class A beta-lactamase (blaTEM) gene partial cds
OP806969.1:103-804 Enterobacter cloacae complex sp. strain CREC-29 TEM family class A beta-lactamase (blaTEM) gene partial cds
CP104331.1:102264-102952 Escherichia coli strain THB42-F3 plasmid pHB42-F3 complete sequence
CP104327.1:103094-103782 Escherichia coli strain THB42-F1 plasmid pHB42-F1 complete sequence
CP091641.1:164093-164794 Salmonella enterica strain 965 plasmid p965-MDR complete sequence
CP091605.1:79529-80230 Salmonella enterica strain 1902 plasmid p1902-MDR complete sequence
ON934556.1:94768-95469 Escherichia coli plasmid p22Q34-1 complete sequence
ON934554.1:41018-41719 Escherichia coli plasmid p19A20-1 complete sequence
ON934552.1:34776-35477 Escherichia coli plasmid p13QT31-1 complete sequence
ON934551.1:25940-26641 Escherichia coli plasmid p13QT11 complete sequence
CP113538.1:4300349-4301050 Salmonella enterica strain XSK chromosome complete genome
CP102709.1:19692-20393 Escherichia coli strain 9A-4-9 plasmid pE2899 complete sequence
CP102703.1:69541-70242 Escherichia coli strain 9A-2-7 plasmid pE2899 complete sequence
OP038301.1:5063-5764 Shigella sonnei strain 201605743 plasmid p201605743-4 complete sequence
OP038293.1:5064-5765 Shigella sonnei strain 202102843 plasmid p202102843-4 complete sequence
OP038282.1:19282-19983 Shigella sonnei strain 201908234 plasmid p201908234-5 complete sequence
OP038277.1:5063-5764 Shigella sonnei strain 201809101 plasmid p201809101-6 complete sequence
CP085199.1:230009-230710 Klebsiella quasipneumoniae strain NDM-101 plasmid unnamed2 complete sequence
CP110375.1:4112688-4113389 Proteus mirabilis strain NYP6 chromosome complete genome
CP110355.1:145669-146370 Enterobacter hormaechei strain ECC2783 plasmid pECC2783 a complete sequence
CP110404.1:2098516-2099217 Shigella sonnei strain S17BD05200 chromosome complete genome
100
CP110195.1:19822-20523 Klebsiella pneumoniae strain FK563 plasmid p2FK563 complete sequence
CP110194.1:7136-7837 Klebsiella pneumoniae strain FK563 plasmid p1FK563 complete sequence
LC735985.1:2645-3346 Citrobacter freundii 12CC1 plasmid p12CC1 CTX-M-3 DNA complete sequence
CP075592.1:75968-76669 Klebsiella pneumoniae strain KpST17-2177 plasmid pKp2177 1 complete sequence
CP027866.1:21593-22294 Klebsiella pneumoniae subsp. pneumoniae strain CRKP18622 plasmid pCRKP18622-KPC complete sequence
CP027802.1:21769-22470 Klebsiella pneumoniae subsp. pneumoniae strain VH1-2 plasmid pVH1-2-KPC complete sequence
CP109601.1:2709623-2710324 Escherichia coli strain TL-14 chromosome complete genome
CP100105.1:29611-30312 Klebsiella pneumoniae strain HSKP33 plasmid pHSKP33-3 complete sequence
CP100073.1:81197-81898 Klebsiella pneumoniae strain RJKP41 plasmid pRJKP41-1 complete sequence
CP080592.1:97192-97893 Cronobacter sakazakii strain Crono-589 plasmid pCrono589-1 complete sequence
CP099523.1:20245-20946 Klebsiella pneumoniae strain SZS128 plasmid pSZS128-Hv-MDR complete sequence
CP104369.1:90099-90800 Salmonella enterica strain PNUSAS048232 plasmid unnamed1 complete sequence
CP104335.1:1184669-1185370 Acinetobacter baumannii strain 2021CK-01408 chromosome complete genome
CP104350.1:1184669-1185370 Acinetobacter baumannii strain 2021CK-01333 chromosome complete genome
CP029683.1:41835-42536 Salmonella sp. FORC89 plasmid pFORC89 complete sequence
ON777850.1:242267-242968 Escherichia coli strain EC600 plasmid p15WZ61-Vir-IncN complete sequence
ON777849.1:12717-13418 Klebsiella pneumoniae subsp. pneumoniae strain 15WZ-61 plasmid p15WZ61-IncN complete sequence

0.050

Appendix 5: Stenotrophomonas maltophilia strain HK 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA intergenic spacer, complete sequence; and 23S ribosomal RNA gene, partial sequence.

GenBank: OP422244.1

```
LOCUS       OP422244               628 bp    DNA     linear   BCT 18-SEP-2022
DEFINITION  Stenotrophomonas maltophilia strain HK 16S ribosomal RNA gene,
            partial sequence; 16S-23S ribosomal RNA intergenic spacer, complete
            sequence; and 23S ribosomal RNA gene, partial sequence.
ACCESSION   OP422244
VERSION     OP422244.1
KEYWORDS    .
SOURCE      Stenotrophomonas maltophilia
  ORGANISM  Stenotrophomonas maltophilia
            Bacteria; Pseudomonadota; Gammaproteobacteria; Xanthomonadales;
            Xanthomonadaceae; Stenotrophomonas; Stenotrophomonas maltophilia
            group.
REFERENCE   1  (bases 1 to 628)
  AUTHORS   Subhi,H.T. and Hamad,H.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (12-SEP-2022) Biology, Koya University, Danielle
            Mitterrand Boulevard, Erbil, Koya KOY45, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..628
                     /organism="Stenotrophomonas maltophilia"
                     /mol_type="genomic DNA"
                     /strain="HK"
                     /isolation_source="Erbil hospitals"
                     /host="Homo sapiens"
                     /db_xref="taxon:40324"
     misc_RNA        <1..>628
                     /note="contains 16S ribosomal RNA, 16S-23S ribosomal RNA
                     intergenic spacer, and 23S ribosomal RNA"
ORIGIN
        1 agccgtatcg gaaggtgcgg ctggatcacc tccttttgag caaagacagc atcgtcctgt
       61 cgggcgtctt cacaaagtac ctgcattcag agaatcacaa cggccaggcc gctgtgagag
      121 tccttttgg ggccttagct cagctgggag agcacctgct ttgcaagcag ggggtcgtcg
      181 gttcgatccc gacaggctcc accacgtttg agcatgttcc ggaaagtatt tccgggtctg
      241 tagctcaggt ggttagagcg cacccctgat aagggtgagg tcggtagttc gagtctaccc
      301 agacccacca ttctctgaat gacgcataca atcgatcttt atacgcatca gcactgtggc
      361 tggtacgtgt tcttttaaaa cttgtgacgt agcgagcgtt tgagatgttc tatcagacgt
      421 gtcgtgaggc taaggcgaga gacgcaagtc tctttattga ttgagtcgtt atattcgtat
      481 ccgggctttg tacccccggg tcatatgtaa cccaaggcaa cttgcggtta tatggtcaag
              601        cgaataagcg cacacggtgg atgccttggc ggtcagaggc gatgaaggac gtggcagcct
      601 gcgaaaagta tcggggagct ggcaacaa
```

Appendix 6: Stenotrophomonas maltophilia strain HK OXA-23 family carbapenem-hydrolyzing class D beta-lactamase (blaOXA) gene, partial cds.

GenBank: OP595162.1

LOCUS OP595162 574 bp DNA linear BCT 10-NOV-2022
DEFINITION Stenotrophomonas maltophilia strain HK OXA-23 family
carbapenem-hydrolyzing class D beta-lactamase (blaOXA) gene,
partial cds.
ACCESSION OP595162
VERSION OP595162.1
KEYWORDS .
SOURCE Stenotrophomonas maltophilia
ORGANISM Stenotrophomonas maltophilia
Bacteria; Pseudomonadota; Gammaproteobacteria; Xanthomonadales;
Xanthomonadaceae; Stenotrophomonas; Stenotrophomonas maltophilia
group.
REFERENCE 1 (bases 1 to 574)
AUTHORS Subhi,H.T. and Hamad,H.M.
TITLE Direct Submission
JOURNAL Submitted (03-OCT-2022) Biology, Koya university, Kurdistan Region,
Koya, Erbil KOY45, Iraq
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..574
/organism="Stenotrophomonas maltophilia"
/mol_type="genomic DNA"
/strain="HK"
/isolation_source="Covid 19 patient sputum"
/db_xref="taxon:40324"
/country="Iraq"
/collection_date="2022"
gene complement(<1..>574)
/gene="blaOXA"
CDS complement(<1..>574)
/gene="blaOXA"
/note="OXA-23 carbapenemase"
/codon_start=3
/transl_table=11
/product="OXA-23 family carbapenem-hydrolyzing class D
beta-lactamase"
/protein_id="UZD11043.1"
/translation="VQHNLINETPSQIVQGHNQVIHQYFDEKNTSGVLVIQTDKKINL
YGNALSRANTEYVPASTFKMLNALIGLENQKTDINEIFKWKGEKRSFTAWEKDMTLGE
AMKLSAVPVYQELARRIGLDLMQKEVKRIGFGNAEIGQQVDNFWLVGPLKVTPIQEVE
FVSQLAHTQLPFSEKVQANVKNMLLLEESNG"
ORIGIN
1 ccattactct cttctaaaag aagcatattt tttacattag cctgcacttt ttcactaaat
61 ggaagctgtg tatgtgctaa ttgggaaaca aactctacct cttgaatagg cgtaaccttt
121 aatggtccta ccaaccagaa attatcaacc tgctgtccaa tttcagcatt accgaaacca
181 atacgtttta cttctttttg catgagatca agaccgatac gtcgcgcaag ttcctgatag
241 actgggactg cagaaagctt catggcttct cctagtgtca tgtctttttc ccaagcggta
301 aatgaccttt tctcgccctt ccatttaaat atttcattaa tatccgtttt ctggttctcc
361 aatccgatca gggcattcaa cattttaaat gtagaggctg gcacatattc tgtatttgcg
421 cggcttagag cattaccata tagattaatt tttttatctg tttgaataac cagcacacct
481 gaggtgtttt tttcatcaaa gtattgatga atcacctgat tatgtccttg aacaatctga
541 ctcggggttt catttattaa attatgctga accg

Appendix 7: Stenotrophomonas maltophilia strain HK NDM family subclass B1 metallo-beta-lactamase (blaNDM) gene, partial cds.

GenBank: OP595163.1

```
LOCUS       OP595163               577 bp    DNA     linear   BCT 10-NOV-2022
DEFINITION  Stenotrophomonas maltophilia strain HK NDM family subclass B1
            metallo-beta-lactamase (blaNDM) gene, partial cds.
ACCESSION   OP595163
VERSION     OP595163.1
KEYWORDS    .
SOURCE      Stenotrophomonas maltophilia
  ORGANISM  Stenotrophomonas maltophilia
            Bacteria; Pseudomonadota; Gammaproteobacteria; Xanthomonadales;
            Xanthomonadaceae; Stenotrophomonas; Stenotrophomonas maltophilia
            group.
REFERENCE   1  (bases 1 to 577)
  AUTHORS   Subhi,H.T. and Hamad,H.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (03-OCT-2022) Biology, Koya university, Kurdistan Region,
            Koya, Erbil KOY45, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
                     /strain="HK"
                     /isolation_source="Covid 19 patient sputum"
                     /db_xref="taxon:40324"
                     /country="Iraq"
                     /collection_date="2022"
     gene            <1..>577
                     /gene="blaNDM"
                     /note="metallo-beta-lactamase NDM"
     CDS             <1..>577
                     /gene="blaNDM"
                     /codon_start=3
                     /transl_table=11
                     /product="NDM family subclass B1 metallo-beta-lactamase"
                     /protein_id="UZD11044.1"
                     /translation="RQLAPNVWQHTSYLDMPGFGAVASNGLIVRDGGRVLVVDTAWTD
                     DQTAQILNWIKQEINLPVALAVVTHAHQDKMGGMDALHAAGIATYANALSNQLAPQEG
                     MVAAQHSLTFAANGWVEPATAPNFGPLKVFYPGPGHTSDNITVGIDGTDIAFGGCLIK
                     DSKAKSLGNLGDADTEHYAASARAFGAAFPKA"
ORIGIN
        1 tccgccagct cgcaccgaat gtctggcagc acacttccta tctcgacatg ccgggtttcg
       61 gggcagtcgc ttccaacggt ttgatcgtca gggatggcgg ccgcgtgctg gtggtcgata
      121 ccgcctggac cgatgaccag accgcccaga tcctcaactg gatcaagcag gagatcaacc
      181 tgccggtcgc gctggcggtg gtgactcacg cgcatcagga caagatgggc ggtatggacg
      241 cgctgcatgc ggcggggatt gcgacttatg ccaatgcgtt gtcgaaccag cttgccccgc
      301 aagaggggat ggttgcggcg caacacagcc tgactttcgc cgccaatggc tgggtcgaac
      361 cagcaaccgc gcccaacttt ggcccgctca aggtatttta ccccggcccc ggccacacca
      421 gtgacaatat caccgttggg atcgacggca ccgacatcgc ttttggtggc tgcctgatca
      481 aggacagcaa ggccaagtcg ctcggcaatc tcggtgatgc cgacactgag cactacgccg
      541 cgtcagcgcg cgcgtttggt gcggcgttcc ccaaggc
```

Appendix 8: A. baumannii strain HK19 NDM family subclass B1 metallo-beta-lactamase (blaNDM) gene, partial cds.

GenBank: OP572243.1

```
LOCUS       OP572243               603 bp    DNA     linear   BCT 10-NOV-2022
DEFINITION  A. baumannii strain HK19 NDM family subclass B1
            metallo-beta-lactamase (blaNDM) gene, partial cds.
ACCESSION   OP572243
VERSION     OP572243.1
KEYWORDS    .
SOURCE      Acinetobacter baumannii
  ORGANISM  Acinetobacter baumannii
            Bacteria; Pseudomonadota; Gammaproteobacteria; Moraxellales;
            Moraxellaceae; Acinetobacter; Acinetobacter calcoaceticus/baumannii
            complex.
REFERENCE   1  (bases 1 to 603)
  AUTHORS   Subhi,H.T. and Hamad,H.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (29-SEP-2022) Biology, Koya university, Kurdistan Region,
            Koya, Erbil KOY45, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
                     /strain="HK19"
                     /isolation_source="Covid 19 patient sputum"
                     /db_xref="taxon:470"
                     /country="Iraq"
                     /collection_date="2022"
                     /note="carbapenems resistance"
     gene            <1..>603
                     /gene="blaNDM"
     CDS             <1..>603
                     /gene="blaNDM"
                     /EC_number="3.5.2.6"
                     /codon_start=2
                     /transl_table=11
                     /product="NDM family subclass B1 metallo-beta-lactamase"
                     /protein_id="UZD11041.1"
                     /translation="RQLAPNVWQHTSYLDMPGFGAVASNGLIVRDGGRVLLVDTAWTD
                     DQTAQILNWIKQEINLPVALAVVTHAHQDKMGGMDALHAAGIATYANALSNQLAPQEG
                     LVAAQHSLTFAANGWVEPATAPNFGPLKVFYPGPGHTSDNITVGIDGTDIAFGGCLIK
                     DSKAKSLGNLGDADTEHYAASARAFGAAFPKASMIVMSHS"
ORIGIN
        1 ccgccagctc gcaccgaatg tctggcagca cacttcctat ctcgacatgc cgggtttcgg
       61 ggcagtcgct tccaacggtt tgatcgtcag ggatggcggc cgcgtgctgt tggtcgatac
      121 cgcctggacc gatgaccaga ccgcccagat cctcaactgg atcaagcagg agatcaacct
      181 gccggtcgcg ctggcggtgg tgactcacgc gcatcaggac aagatgggcg gtatggacgc
      241 gctgcatgcg gcggggattg cgacttatgc caatgcgttg tcgaaccagc ttgccccgca
      301 agaggggctg gttgcggcgc aacacagcct gactttcgcc gccaatggct gggtcgaacc
      361 agcaaccgcg cccaactttg gcccgctcaa ggtattttac cccggccccg gccacaccag
      421 tgacaatatc accgttggga tcgacggcac cgacatcgct tttggtggct gcctgatcaa
      481 ggacagcaag gccaagtcgc tcggcaatct cggtgatgcc gacactgagc actacgccgc
      541 gtcagcgcgc gcgtttggtg cggcgttccc caaggccagc atgatcgtga tgagccattc
      601 cga
```

Appendix 9: A. baumannii strain HK22 TEM family class A beta-lactamase (blaTEM) gene, partial cds.

GenBank: OP572244.1

```
LOCUS       OP572244               702 bp    DNA     linear   BCT 10-NOV-2022
DEFINITION  A. baumannii strain HK22 TEM family class A
            beta-lactamase (blaTEM) gene, partial cds.
ACCESSION   OP572244
VERSION     OP572244.1
KEYWORDS    .
SOURCE      Acinetobacter baumannii
  ORGANISM  Acinetobacter baumannii
            Bacteria; Pseudomonadota; Gammaproteobacteria; Moraxellales;
            Moraxellaceae; Acinetobacter; Acinetobacter calcoaceticus/baumannii
            complex.
REFERENCE   1  (bases 1 to 702)
  AUTHORS   Subhi,H.T. and Hamad,H.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (29-SEP-2022) Biology, Koya university, Kurdistan Region,
            Koya, Erbil KOY45, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /isolation_source="Covid 19 patient sputum"
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                     /country="Iraq"
                     /collection_date="2022"
                     /note="beta-lactam resistance"
     gene            <1..>702
                     /gene="blaTEM"
     CDS             <1..>702
                     /gene="blaTEM"
                     /codon_start=1
                     /transl_table=11
                     /product="TEM family class A beta-lactamase"
                     /protein_id="UZD11042.1"
                     /translation="EDQLGARVGYIELDLNSGKILESFRPEERFPMMSTFKVLLCGAV
                     LSRVDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVRELCSAAITMSDNTAANLL
                     LTTIGGPKELTAFLHNMGDHVTRLDRWEPELNEAIPNDERDTTMPAAMATTLRKLLTG
                     ELLTLASRQQLIDWMEADKVAGPLLRSALPAGWFIADKSGAGERGSRGIIAALGPDGK
                     PSRIVVIYTTGSQATM"
ORIGIN
        1 gaagatcagt tgggtgcacg agtgggttac atcgaactgg atctcaacag cggtaagatc
       61 cttgagagtt ttcgccccga agaacgtttt ccaatgatga gcacttttaa agttctgcta
      121 tgtggtgcgg tattatcccg tgttgacgcc gggcaagagc aactcggtcg ccgcatacac
      181 tattctcaga atgacttggt tgagtactca ccagtcacag aaaagcatct tacggatggc
      241 atgacagtaa gagaattatg cagtgctgcc ataaccatga gtgataacac tgctgccaac
      301 ttacttctga caacgatcgg aggaccgaag gagctaaccg cttttttgca caacatgggg
      361 gatcatgtaa ctcgccttga tcgttgggaa ccggagctga atgaagccat accaaacgac
      421 gagcgtgaca ccacgatgcc tgcagcaatg gcaacaacgt tgcgcaaact attaactggc
      481 gaactactta ctctagcttc ccggcaacaa ttaatagact ggatggaggc ggataaagtt
      541 gcaggaccac ttctgcgctc ggcccttccg gctggctggt ttattgctga taaatctgga
      601 gccggtgagc gtgggtctcg cggtatcatt gcagcactgg ggccagatgg taagccctcc
      661 cgtatcgtag ttatctacac gacggggagt caggcaacta tg
```

Appendix 10: Result of VITEK 2 compact system.

bioMérieux Customer:

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Microbiology Chart Report

Printed Jan 24, 2022 11:02 CST

Patient Name: khdhr hamad, hadi
Location:
Lab ID: hadi 55

Patient ID: 26869
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Acinetobacter baumannii

Source: sputum

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.80 hours	**Status:** Final
Selected Organism	99% Probability **Bionumber:**	**Acinetobacter baumannii** 0241011103500212	
ID Analysis Messages			

Susceptibility Information	**Analysis Time:** 7.50 hours				**Status:** Final
Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**Interpretation**
Piperacillin	>= 128	R	Netilmicin	>= 32	R
Piperacillin/Tazobactam	>= 128	R	Tobramycin	>= 16	R
Ceftazidime	>= 64	R	Ciprofloxacin	>= 4	R
Cefepime	>= 32	R	Levofloxacin	>= 8	R
Aztreonam			Tetracycline	>= 16	R
Imipenem	>= 16	R	Tigecycline	2	S
Meropenem	>= 16	R	Colistin	<= 0.5	S
Amikacin			Trimethoprim/Sulfamethoxazole	>= 320	R
Gentamicin	>= 16	R			

+= Deduced drug *= AES modified **= User modified

AES Findings	
Confidence:	Consistent

Appendix 11: Biochemical results by using VITEK 2 Compact system.

bioMérieux Customer:
System #:

Laboratory Report

Printed Jan 24, 2022 11:02 CST
Printed by: Labadmin

Patient Name: khdhr hamad, hadi
Isolate: hadi 55-1 (Approved)

Patient ID: 26869

Card Type: GN Bar Code: 2411684103114649 Testing Instrument: 000019E1EC77 (18750)
Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 0241011103500212
Organism Quantity:

Selected Organism: Acinetobacter baumannii

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	+	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	+	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	+			

Installed VITEK 2 Systems Version: 08.01
MIC Interpretation Guideline: Global CLSI-based
AES Parameter Set Name: Global CLSI-based+Phenotypic

Therapeutic Interpretation Guideline: PHENOTYPIC
AES Parameter Last Modified: Apr 13, 2020 19:29 CDT

Page 2 of 2

Appendix 12: VITEK 2compact system results for all *A.baumannii*.

Well	Test	Mnemonic	Result	(+)%
2	Ala-Phe-Pro-ARYLAMIDASE	APPA	–	0
3	ADONITOL	ADO	–	0
4	L-Pyrrolydonyl-ARYLAMIDASE	PyrA	–	0
5	L-ARABITOL	lARL	–	0
7	D-CELLOBIOSE	dCEL	V	93.8
9	BETA-GALACTOSIDASE	BGAL	–	0
10	H2S PRODUCTION	H2S	–	0
11	BETA-N-ACETYL-GLUCOSAMINIDASE	BNAG	–	0
12	Glutamyl Arylamidace pNA	AGLTp	V	87.5
13	D-GLUCOSE	dGLU	V	93.8
14	GAMMA-GLUTAMYL-TRANSFERASE	GGT	–	0
15	FERMENTATION/ GLUCOSE	OFF	–	0
17	BETA-GLUCOSIDASE	BGLU	–	0
18	D-MALTOSE	dMAL	–	0
19	D-MANNITOL	dMAN	–	0
20	D-MANNOSE	dMNE	V	93.8
21	BETA-XYLOSIDASE	BXYL	–	0
22	BETA–Alanine arylamidace pNA	BAlap	–	0
23	L–Proline ARYLAMIDASE	ProA	V	18.8
26	LIPASE	LIP	–	0
27	PALATINOSE	PLE	–	0
29	Tyrocine ARYLAMIDASE	TyrA	V	81.3
31	UREASE	URE	V	43.8

32	D-SORBITOL	dSOR	–	0
33	SACCHAROSE/SUCROSE	SAC	–	0
34	D-TAGATOSE	dTAG	–	0
35	D-TREHALOSE	dTRE	–	0
36	CITRATE (SODIUM)	CIT	V	87.5
37	MALONATE	MNT	V	93.8
39	5-KETO-D-GLUCONATE	5KG	–	0
40	L–LACTATE alkalinication	lLATk	V	93.8
41	ALPHA-GLUCOSIDASE	AGLU	–	0
42	SUCCINATE alkalinication	SUCT	V	93.8
43	Beta-N-ACETYL-GALACTOSAMINIDAS E	NAGA	–	0
44	ALPHA-GALACTOSIDASE	AGAL	–	0
45	PHOSPHATASE	PHOS	–	0
46	Glycine ARYLAMIDASE	GlyA	–	0
47	ORNITHINE DECARBOXYLASE	ODC	–	0
48	LYSINE DECARBOXYLASE	LDC	–	0
53	L–HISTIDINE accimilation	lHISa	V	43.8
56	COUMARATE	CMT	+	100
57	BETA-GLUCORONIDASE	BGUR	–	0
58	O/129 RESISTANCE (comp.vibrio.)	O129R	+	100
59	Glu-Gly-Arg-ARYLAMIDASE	GGAA	–	0
61	L–MALATE accimilation	lMLTa	V	37.5
62	ELLMAN	ELLM	V	12.5
64	L–LACTATE accimilation	lLATa	V	56.3

UNIVERSITY

کۆماری فیدراڵی عێراق

حکومەتی هەرێمی کوردستان

وەزارەتی خوێندنی باڵاو توێژینەوەی زانستی

زانکۆی کۆیە

ناسینەوەی گەردی هەندێک لە بۆهێڵەکانی بەرگری دژەزیندەیی بەکتریای بۆمانی کەجیاکراوەتنەوە لە نەخۆشەکانی هەولێر/هەرێمی کوردستان-عێراق

ماستەرنامەیەکە پێشکەشی فاکەڵتی زانست و تەندروستی کراوە
لە زانکۆی کۆیە وەك بەشێك لەپێداویستیەکانی بەدەستهێنانی بڕوانامەی ماستەرلە بواری زیندەزانی

لەلایەن

هەژیر مولود حمد

بەکالۆریۆس لە زیندەزانی

فاکەڵتی زانست و تەندروستی / زانکۆی کۆیە

بەسەرپەرشتی: پ.ی.د حنان طارق صبحی

٢٧٢٢

پوختە

لەم چەند ساڵەی دواییدا، بەکتریای جۆری *A. baumannii* بو بەیەکێک لە هۆکارە کاریگەرەکان بۆ توشبوون بە نەخۆشیە کووشندەکانی مرۆڤ، بە تایبەتی دوای دەرکەوتنی ڤایرۆسی کۆرۆنا کە ناسراوە بە *covid19*. هێزی شاراوەی بەکتریای جۆری *A. baumannii* هەیەتی، ئەگەڕێتەوە بۆ تواناى وەرگرتنی ژمارەیەکی زۆر و نادیاری بۆهێڵەکانی (جینەکانی) بەرگری دژی دژەزیندەییەکان.

بۆیە، لەم توێژینەوەیەدا کۆمەڵێک بۆهێلی تایبەت بە بەرگری دژی دژەزیندەیەکان شیکارکران بە پشتبەستن بەو ئەنجامە ڕۆکەشیانەی (بەرگری دژی دژەزیندەی و تایبەتمەندیەکانی تر) کە لە کاتی جیاکردنەوەی نموونەی بەکتریاکان لە نەخۆشەکانی هەندێک لە نەخۆشخانەکانی هەولێر بەردەست بوون.

نمونەکانی بەکتریای *A. baumannii* کە وەرگیراون لە نەخۆشەکان، بە بەکارهێنانی چەند ڕێگەیەکی جیاواز لێکۆڵینەوەیان لەسەر کرا، کە ئەمانە دەگرێتەوە: پشکنینی کیمیای ژیانی ئاسایی و بەکارهێنانی جیهازی VITEK compact 2 system و لە ڕووی هێڵیشەوە بەکارهێنانی ڕێگەی Polymer Change Reaction (PCR) بۆ دیاریکردن و شیکاریکردنی هێڵی 16S-23S rRNA intragenic spacer gene (ITS) کە بۆهێڵێکی تایبەتە بەو جۆرە بەکتریایە، وە دواتر شیکارکردنی ڕیزبەندی نیوکیلۆتایدەکان لە ڕێگەی بەکارهێنانی تەکنیکی Sanger Sequence، وە هەروەها، دیارکردنی تایبەتمەندیەکانی نموونەی بەکتریاکان لە ڕێگەی پشکنینەکانی کیمیای ژیانی، دیاریکردنی بوونی پلازمید، دروستکردنی جۆری بایۆفیلم لە ڕێگەی بەکارهێنانی (microtiter)، دیاریکردنی بەرگری دژی دژەزیندەیی بە ڕێگەی VITEK compact 2 system و PCR. لەم توێژینەوەیەدا بیست و دوو بۆهێڵی بەرگری دژی دژەزیندەییەکان دەستنیشانکران بۆ دیاریکردنی ئەم بۆهێڵانە لە ناو نمونەی بەکتریا جیاکراوەکان، کە ئەم بۆهێڵانە دەگرێتەوە: بۆهێڵەکانی بێتا-لاکتامەیس: bla_{OXA-51}, bla_{OXA-58}, bla_{OXA-23}, bla_{TEM}, bla_{NDM}, and bla_{SHV}، بۆهێڵەکانی ئەمینۆگلایکۆساید: *aph(3')-VI, aacA4, aadB, strA* and *strB*، بۆهێڵەکانی سەلفۆنامید: *sul I* and *sul II*، بۆهێڵی دەرپەڕاندنی فرە دژە زیندەیی *adeB*، بۆهێڵەکانی تێتراسایکلین: *tetA* and *tetB*، بۆهێڵەکانی ماکرۆلید *msr(E), mph(E)* and *erm 42*، بۆهێڵی تریمیثۆپریم *dhfr1*، بۆهێڵەکانی فلۆرۆقوینۆلین: *parC* and *gyrA*.

(٥٧٠) نموونە وەرگیران لە نەخۆشەکانی نەخۆشخانە جیاوازەکانی شاری هەولێر کە ئەمانە دەگرێتەوە: خوێن، میز، پیسایی، کێم و چڵک، سواپ وە شلەکانی جەستە. ٢٣ بەکتیریای *A. baumannii* جیاکرایەوە و دەستنیشانکرا کرا بە بەکارهێنانی پشکنینی کیمیای ژیان و VITEK 2 compact system. لە نێو (١٠٠) نمونە (٢٨.٢١٪) توشبووی ڤایرۆسی *covid-19* بوون، کە ١٣٪ میان توشی بەکتریای *A. baumannii* بوون. هەموو بەکتریا جیاکراوەکان هەڵگری پلازمید بوون، بەڵام، هیچ کامیان دروستکەری بایۆفیلمی بەهێز نەبوون. لەگەڵ ئەوەی ٢.٤١٪ ی بەکتریا جیاکراوەکان دروستکەری بایۆفیلمی ناوەندی بوون و ٣.٣٥٪ یان توانای دروستکردنی بایۆفیلمی لاوازیان هەبوو وە ٦.١٧٪ یان توانای دروستکردنی بایۆفیلمیان نەبوو. پلازمید و کرۆمۆسۆمی بەکتریاکان هەردووکیان هەڵگری بۆهێڵی دژ بە دژەزیندەییەکان بوون. وە هەموو بەکتریایەکی جیاکراوە بە لایەنی کەم حەوت بۆهێڵی بەرگری دژ بە دژە زیندەییەکانیان هەڵگرتبوو. لەگەڵ

ئەوەشدا ئەمە یەکەم توێژینەوەیە کە لێکۆڵینەوە لەم ڕێژە زۆرەی بۆهێڵێ دژ بە دژە زیندەییەکان دەکات لە ناو پلاسمید و گرۆمۆسۆمی بەکتریای جۆری *A. baumannii* بەشێوەیەکی ئیپیدیمیکلی هەولێر/عراق، بۆهێڵی bla_{TEM} لە ناو هەموو بەکتریا جیاکراوەکان دەستنیشانکراوە کە لە نێوان بەکتریاکاندا بڵاوبۆتەوە، وە بۆهێڵی *aadB* لەلایەن هیچ کام لە بەکتریاکانەوە هەڵنەگیرابوو. بەشێکی زۆر لە بۆهێڵەکان کە لە ناو کرۆمۆسۆمی بەکتریاکە دەستنیشاننەکران، بەڵام لە ناو پلازمیدەکایان دەستنیشانکران. سەرەڕای ئەوەش، بۆهێڵێکی نوێی کاربۆپێنیمەیس دەستنیشانکرا کە لە پلازمیدی توخمێکیتری بەکتریاوە وەرگیراوە، وەک و *Klebsiella pneumonia* و *E.coli* کە لەگەڵ بەکتریای *A. baumannii* تووشی هەمان نەخۆش بوون لەو کەیسانەی کە لەم توێژینەوەیەدا لێکۆڵینەوەی لەسەر کراوە. ئەو بۆ هێڵە نوێیانەی کە لەم توێژینەوەیەدا بەدەستهاتوون ئەمانەن: بۆهێڵی bla_{NDM} کە بەم ناونیشانی *A. baumannii* strain carbapenems resistance HK19 و ژمارەی ئەکسیشنی OP572243 لە بانکی بۆهێڵی لە ماڵپەڕێ NCBI داخیلکراوە، وە بۆهێڵی bla_{TEM} کە بە ناونیشانی *A. baumannii* strain beta-lactam resistance HK22 وە بە ژمارەی ئەکسیشنی OP572244 داخیل کراوە، هەروەها لەم توێژینەرەدا سترەینێکی نوێی *Stenotrophomones maltophilia* دەسکەوتووە کە بە ژمارەی ئەکسیشنەکەی OP422244 یە، کە دوو بۆهێڵی نوێی بێتا-لاکتامەیسیان هەڵگرتووە کە (bla_{NDM} و bla_{OXA-23}) ن بە ژمارەی ئەکسشنی OP595162 و OP595163. ٣.٩٣٪ بەکتریا جیاکراوەکان لەم توێژینەوەیەدا میتابۆلیزمی شەکریان بەکارهێناوە وە ١٠٠٪ بەکتریا جیاکراوەکان میتابۆلیزمی کیومیرەیتیان بەکار هێناوە.

ئەنجامە گرینگەکان لەم توێژینەوەیەدا نیشانی دەدەن کە پلاسمید گرینگی زۆری هەیە لە سترەینەکانی *A. baumannii* بۆ دەستکەوتنی بۆهێلی بەرگری لە دژی دژەبەکتریاکان، هەروەها، ریچکەی زیندەچالاکی لە هەموو بەکتریا جیاکراوەکان بریتیبووە لە ڕێچکەی زیندەچالاکی کیومیرەیت، لەگەڵ ئەوەی هەموو بەکتریا جیاکراوەکان ڕێچکەی زیندەیی جیاوازیان بەکاردێنن، بەڵام، هەمووشیان ڕێچکەی زیندەچالاکی کیومیرەیتیان بەکارهێناوە بۆ مانەوە لە ژیان.

جمهورية العراق الفدرالة
حكومة إقليم كوردستان
وزارة التعليم العالي والبحث العلمي
جامعة كوية

UNIVERSITY

التشخيص الجزيئي عن بعض الجينات المقاومة للمضادات الحيوية لعزلات بكتيريا البومانية من المرضى في أربيل/إقليم كردستان-العراق

رسالة مقدمة الى مجلس كلية العلوم والصحة في جامعة كويه وهي جزء من متطلبات نيل شهادة الماجستير في اختصاص علوم الحياة

من قبل

هەژير مولود حمد

بكالوريوس في علوم الحياة

كلية العلوم والصحة / جامعة كوية

بإشراف : ا. م . د. حنان طارق صبحى

1444

2023

الخلاصة

في الآونة الأخيرة ، ازدادت اعداد بكتريا*A. baumannii* للمقاومة المتعددة للأدوية من تأثيرها على وحدات العناية المركزة والعناية بالمرضى على وجه التحديد بعد جائحة Covid19. وبما ان بكتريا *A. baumannii* تعتمد على الأعداد غير المعلومة لجينات المقاومة المكتسبة, لذلك تم تشخيص وتحديد كل من النمط الظاهري وجينات مقاومة المضادات الحيوية للعزلات البكتيرية التي تم جمعها من المرضى في عدد من مستشفيات أربيل. تمت الدراسة بالتعرف على عزلات *A. baumannii* عن طريق الاختبارات الكيميائية الحياتية ونظام VITEK Compact 2 بلاضافة الى النمط الجيني باستخدام تفاعل تغيير البوليمر (PCR) للكشف عن الجين المباعد داخل الجين S-23S 16rRNA (ITS) ومن ثم التعرف على التسلسل الجيني باستخدام تقنية Sanger.

تضمنت توصيف العزلات : ١. تحديد الصفات الكيميائية الحيوية. ٢.الكشف عن وجود البلازميدات. ٣. التقدير الكمي لتكوين الأغشية الحيوية باستخدام طريقة (microtitre). ٤. الكشف الظاهري لوجود مقاومة المضادات الحيوية باستخدام نظام VITEK 2. ٥. الكشف الجيني لمقاومة المضادات الحيوية باستخدام تقنية بلمرة PCR.

تم البحث عن ٢٢جين لمقاومة المضادات الحيوية وهي :جينات بيتا لاكتاماز وتشمل bla_{OXA-51}و bla_{OXA-58} و bla_{OXA-23} و bla_{TEM} و bla_{NDM} و bla_{SHV}. جيناتaminoglycoside وتشمل *aph(3')-VI*، *aacA4*، *aadB*، *strA* و *strB*. جينات السلفوناميد وتشمل *sul I* و *sul II*. جين تدفق الأدوية المتعددة وتشمل *adeB* ، جينات التتراسيكلين وتشمل جينات *tetA* و *tetB* وجينات macrolide وتشمل *msr (E)*، *mph (E)* و *erm 42*، وجين Trimethoprim وتشمل *dhfr1* ، وجينات الفلوروكينولونات وتشمل *parC* و *gyrA*.

تضمنت الدراسة ٥٧٠ عينة مريض وهي عينات الدم والبلغم والبول والبراز والقيح وسوائل الجسم, وتم عزل وتشخيص ٢٣ (٤.٠٤%) من بكتريا *A. baumannii* عن طريق استخدام الاختبارات البيوكيميائية ونظام VITEK.

من بين ١٠٠ (٢١.٢٨%) عينة من مرضى كوفيد -١٩ ، ١٣ (١٣٪) عزلة كانت *A. baumannii*. جميع عزلات *A. baumannii* كانت حاملة للبلازميد واظهرت عدم تكوين غشاء حيوي قوي biofilm. ومع ذلك ، كان الأغشية الحيوية متوسطة التكوين معنوية عند ٤١.٢٪ ، وضعيف التكوين عند ٣٥.٣٪ ، وغير حيوي التكوين كانت غير معنوية بنسبة ١٧.٦٪.

ظهرت جينات المقاومة في كل من كروموسوم وبلازميد البكتيريا و كانت كل عزلة بكتيرية حاملة لسبعة جينات مقاومة على الأقل. الى جانب انها الدراسة الأولى التي تكتشف عن أوبئة المقاومة الجينية في أربيل / العراق ، اكتشفت ان bla_{TEM} هي الجين المنتشر في جميع العزلات ،وان الجين المقاوم *aadB* غير مشفرة في جميع العزلات.

اضافة الى العديد من جينات المقاومة التي وجدت على بلازميد عزلات البكتريا بدلا من الكروموسوم ، تم اكتشاف تسلسل جيني جديد لجين carbapenemase على كروموسوم البكتريا والذي تبين ان مصدره بلازميدات أجناس بكتيرية مختلفة مثل *Klebsiella pneumonia* و *E.coli* كانت مصاحبة لحالات الاصابة ب *A. baumannii* في هذة الدراسة.

الجينات الحديثة المكشفة في هذة الدراسة هي:

bla_{NDM}، تحت مسمى- *A. baumannii* strain HK19 NDM family subclass B1 metallo-beta-lactamase (bla_{NDM}) gene؛ رقم الانضمام الى بنك الجينات OP572243

bla_{TEM} تحت مسمى: *A. baumannii* strain HK22 TEM family class A beta-lactamase (bla_{TEM}) gene؛ رقم الانضمام الى بنك الجينات OP572244

بالإضافة إلى ذلك ، تم اكتشاف سلالة جديدة من بكتريا *Stenotrophomones maltophilia* رقم الانضمام الى بنك الجينات OP422244 .

اكتسبت السلالة المكتشفة *Stenotrophomones maltophilia* جينات بيتا لاكتاماز جديدة من بكتريا *A baumannii* وهي bla_{NDM}: رقم الانضمام الى بنك الجينات OP595162 و bla_{OXA-23} تحت رقم الانضمام الى بنك الجينات OP595163 . اما مسار التمثيل الغذائي للعزلات كانت عبارة عن مسارالكربوهيدرات بنسبة ٩٣.٣٪ ومسار كومارات coumarate بنسبة ١٠٠٪.

النتائج المهمة في هذه الدراسة هي: اولا الدور الرئيسي للبلازميد في مقاومة المضادات الحيوية.
وثانيا المسار الأيضي في جميع السلالات هو المسار الأيضي الكوماري cumarate ، وعلى الرغم من المسارات الأيضية العديدة في العزلات ولكن جميع السلالات استخدمت مسار الكومارات للمقاومة وللبقاء على قيد الحياة.