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Genetic analysis of Acenitobacter bumannii associated with viral respiratory infections

Noor Qahtan AL Sarraf
Researcher, University / College of Education / Department of Life Sciences

Prof. Dr. Dina Mohammed Raouf Al-Qadisiyah
Researcher, University / College of Education / Department of Life Sciences

***Abstract*---**In this study, samples were collected from Corona patients who were in isolation units for Corona virus patients, where samples were taken (from the nasal passage, mouth and sputum) and the samples were transferred by a liquid nutrient medium to the laboratory and kept at -2 temperature until they were cultivated on the medium of the Maconkey and saved In the incubator for 24 hours to observe the types of bacteria present , The samples were also cultured on Chrom agar Base Acinetobacter bacteria (which is a selective medium).It is difficult to distinguish it phenotypically from other bacteria, The phenotypic detection of A. bumannii bacteria, biochemical tests, and then molecular detection using ITS-specific primer were also performed and molecular detection using SP4-specific primer were also performed . A molecular assay for the CipA gene that encodes for the production of an enzyme complementary-inhibitor and Plg-binding protein of A. baumannii (Cip A) was also performed and the molecular assay for the Peptidase M10, metallopeptidase-specific primer gene was also performe. As the most important virulence factors in A. bumannii, Because the two enzymes played an important role in the pathogenesis of these bacteria. And the result was (20% of the MMP10 gene and 100% of the CipA gene).

***Keywords*---**genetic analysis, associated, respiratory infections.

Introduction

Bacteria cause secondary infections in corona virus patients. *Acinetobacter species* are characterized by the formation of gram-negative, aerobic, non-motile bacilli. It is not fermented to the sugar lactose, its importance has recently emerged as an opportunistic pathogen responsible for many of the Hospital-

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***Corresponding author**: Sarraf, N, Q, A.*
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acquired infections (Nosocomial infection), in addition to their ability to acquire Resistance to life antibiotics, and its ability to adapt the biofilm, which increases the chances of its survival in the environment Hospitals for a long time, which helps my spread (Poirel, *et al*; 2011). *A. baumannii* was first isolated from soil by Dutch bacteriologist Beijerinck in 1911 and described as Micrococcus calcoaceticus. After 50 years, the same bacterium was isolated several times and reported by various names such as Moraxella lwoffi, Alcaligenes hemolysans, Mirococcuscalco-aceticus, and Herellea vaginicola (Asif,*et al*;2018).

They are free throwing organisms that are widely distributed in various environments including soil, water, wastewater, vegetables, animal skins, (Maravić A, *et al*; 2016). They were isolated from various parts of the body of healthy individuals, including the nose, ear, throat, forehead, trachea, vagina and perineum, armpits, vulva, hands and nets of toes and humans (Al Atrouni A et al; 2016). When focusing on secondary infections of the lower respiratory tract of COVID-19 patients, *A. baumannii* was the most common organism (Sharifipour*, et al*; 2020). Secondary infection of *A. baumannii* associated with SARS-CoV-2 infection has also been reported several times in studies during the COVID-19 epidemic including Wuhan (China), France, Spain, Iran, Egypt, New York (USA), Italy and Brazil through the occurrence of secondary infections (mostly lower respiratory tract infections) due to *A. baumannii* (Ripa,*et al;*2021). It has gained the ability to infect not only hospital patients but also the general population in hospitals, mortality rate is 26% up to 43% in intensive care (Greene C *et al*; 2016). Units *A. baumannii* is not a community pathogen, but in immunocompromised individuals and children, it attacks the trachea and can cause bronchiolitis and community-induced tracheobronchitis. It is implicated in community-acquired pneumonia under certain conditions such as smoking, alcoholism, diabetes mellitus, and COPD. In tropical regions of Asia and Australia, *A. baumannii* may be implicated in bloodstream infections in 10%-15%. of cases due to respiratory procedures such as (catheters, tubes, or cannulas within the blood vessels or respiratory system), In 22%-70% of *A. baumannii* infections, the source of infection is still unknown and *A. baumannii* is considered a growing threat to neurosurgery patients. It is responsible for 4% of all meningitis and shunt related infections, with mortality rates of up to 70%, and is responsible for 2.1% of ICU-acquired wound infections. They were pronounced (32%) in casualties from the battlefields of Afghanistan and Iraq. It's also not a usual factor in urinary tract infections (UTIs). However, it can cause infection in elderly patients and patients with prolonged periods of catheter-related infection residing in intensive care units where we contribute 1.6% of total UTIs. May cause endocarditis. Keratitis and ocular inflammation after contact lens use and eye surgery. Bacterial movement contributes to an increased capacity for infection and an increase in the risk of some bacteria (Ayoub Moubareck, et al;2020).

Materials and Methods

Identification of bacterial isolates
Morphologic characteristics

The isolated bacterial isolates were initially diagnosed according to the phenotypic characteristics of the colonies' shape, colour, texture and size, as well as their

ability to degrade red blood cells on a blood agar medium and their ability to ferment lactose sugar on Maconkey medium.

Microscopic characteristics

A portion of the pure colonies at the age of 24 hours were transferred by Loop to a glass slide and a drop of distilled water was placed on it, spread on an area of the slide, then fixed and stained with gram stain.

Extraction of bacterial DNA from samples of corona patients

In this study, DNA was extracted using Presto Mini gDNA Bacteria Kit from Geneaid Company.

Polymerase chain reaction for diagnosing bacteria *Acenitobacter bumannii* and some virulence factors

PCR amplification of DNA was performed by a thermo cycler with a final mixture volume of 25 µl. PCR products were analyzed by 1.5% agarose gel electrophoresis and clarified under UV illumination after staining with ethidium bromide dye. The PCR mixtures and conditions for this PCRTaq®G2Green Master Mix are summarized according to the instructions of the manufacturer Promega in Table (1) which comes with the kit.

Table 1
Diagnosis of *A. bumannii* by 16S-23S ribosomal DNA intergenic spacer region (ITS)

Ingredients	Volume in µl
Master Mix	µL 12.5
Forward Primer	µL 2.5
Reverse Primer	µL 2.5
Template DNA	µL 3
Nuclease free water	µL 4.5
Total volume	µL 25

Table 2
Terms of the interaction cycle for the ITS-specific primer

The gene	Initiator sequence	temperature		Time	Number of cycles
ITS-specific primer	CATTATCACGGTAATTAGTG	PA-F	°C**95**	Min5	1
	AGAGCACTGTGCACTTAAG	PA-R	°C**94**	Min1	30
			°C **55**	Min1	
				Min1	
				Min10	

Table 3

Diagnosis of *A. bumannii* by Sp4

Ingredients	Volume in µl
Master Mix	µL12.5
Forward Primer	µL 2.5
Reverse Primer	µL 2.5
Template DNA	µL 3
Nuclease free water	µL 4.5
Total volume	µL 25

Table 4
Terms of the interaction cycle for the *Sp4*-specific primer

The gene	Initiator sequence		temperature	Time	Number of cycles
Sp4-specific primer	CACGCCGTAAGAGTGCATTA	PA-F	°C**95**	min**5**	**1**
	AACGGAGCTTGTCAGGGTTA	PA-R	°C**94**	Min**1**	**30**
			°C**52**	Min**1**	
			°C 72	Min**1**	
			°C **72**	Min**10**	**1**

Results and Discussion

120 samples were collected from Corona patients present in the isolation unit for Corona patients, and all samples were cultured in Maconkey medium and Acenitobacter bumannii medium as shown in Table (5) below:

Table 5
Types and numbers of bacteria and fungi isolated from 120 infected with coronavirus who were in the isolation unit

	Types of bacteria and fungi	The emergence of isolates	
		the number	The ratio**%**
1	*Staphylococcus aureus*	**27**	**%22.5**
2	*Acenitbacter bumannii*	**24**	**%20**
3	*Streptococcus pneumoniae*	**20**	**%16.66**
4	Gemella morbillorum	**7**	**%5.8**
5	Staphylococcus spp	**6**	**%5**
6	*Streptococcus oralis*	**4**	**%3.33**
7	*Granulicatella adicens*	**3**	**%2.5**
8	*Rothia dentocariosa*	**2**	**%1.66**
9	*Candida spp*	**2**	**%1.66**

The results of the table showed a decrease in the number of natural flora compared to pathogenic bacteria, as this occurs due to the intake of antibiotics, which leads to the death of the natural flora and the survival of pathogenic bacteria due to its resistance to antibiotics. This is consistent with studies that show that common antibiotics have a broad spectrum of activity, which It leads to the random killing of pathogens and natural flora and a disturbance in the balance of microorganisms resulting from treatment with antibiotics, which leads

to severe and frequent cases of complications due to chronic pathogens or due to microorganisms (Eckert, 2011, RuizJ,2021). Where he confirmed in his study that the presence of coronavirus patients in isolation units exposes them to severe consequences of secondary infection, and confirmed that the use of mechanical ventilation produces ventilator-associated pneumonia (VAP), especially with multidrug-resistant bacteria such as A. baumannii, cases have been reported. Infection of *A. baumannii* in COVID-19 patients. As shown (Sharifipour,et al;2020).

Detection of Acenitobacter bumannii by ITS-specific primer

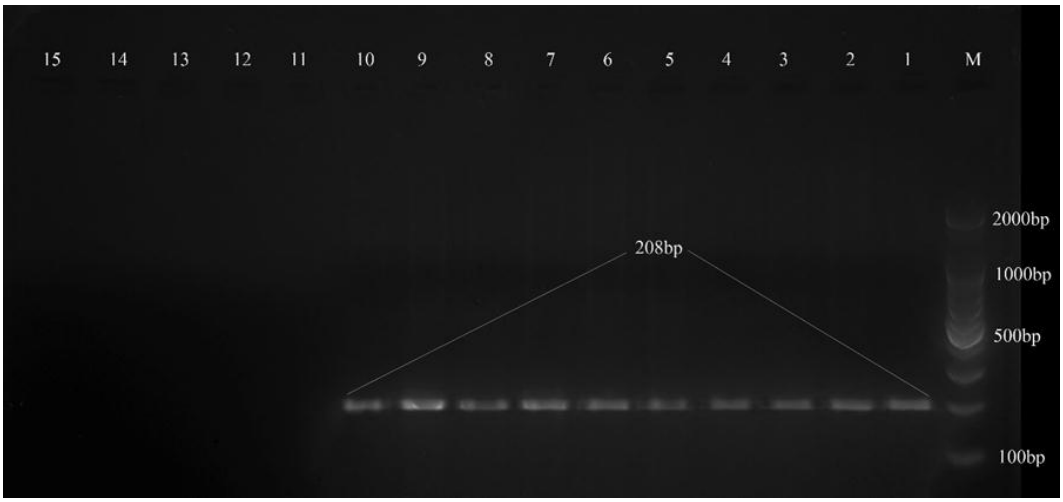

Figure 1. Electrophoresis of the PCR product obtained using the ITS-specific primer gene (208) base pairs of Acenitobacter bumannii isolates. Pathway from (1-10) A. bumannii isolates. Characterized by either pathway M Volume guide (100 base pairs)

According to the result shown above, 20% (24) sample was positive as it was confirmed as Acenitobacter bumannii based on 16S-23S ribosomal DNA intergenic spacer region (16S-23S ribosomal DNA) (ITS).

Detection of Acenitobacter bumannii by the gyrB-(Sp4)-gene

DNA was extracted from 120 samples under study. The results of the amplification of the gene were shown GyrB- (Sp4)-gene that 20% (24) samples were positive for this assay as shown in Figure (2).

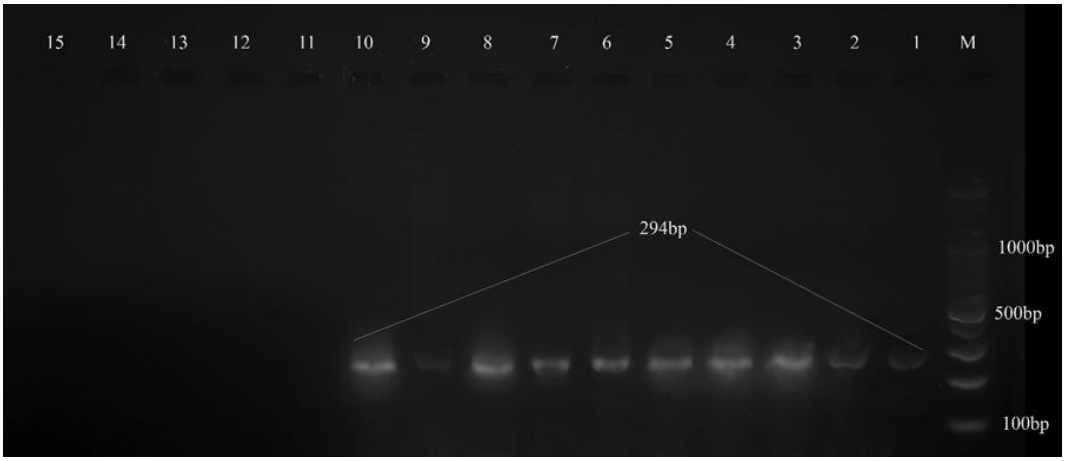

Figure 2. Electrophoresis of the polymerase chain reaction (PCR) product obtained using the gyrB-Sp4 gene (294) base pair of Acenitobacter bumannii isolates. Pathway from (1-10) A. bumannii isolates. Characterized by either pathway M Volume guide (100) base pairs

According to the above result, 20% (24) was a positive sample as it was confirmed as Acenitobacter bumannii based on the gyrB-gene.

Phylogenetic analysis of *Acenitobacter bumannii*

In this study, the degree of similarity between 10 samples of *A. bumannii* bacteria was studied. Through Table No. (4) that shows the similarity ratios between the isolates used in our study and the source bacteria NZ_CP043953.1, where we note the similarity rate between sample No. 1, 3 and 6 with the source bacteria was 99%, and the similarity rate with sample No. 2, 8 and 9 And 10 was 98%, and the percentage of similarity of the source bacteria with sample No. 4, 5 and 7 was 100%, and through that we notice a great similarity between the studied samples and the source bacteria. The similarity ratio was also studied between each of the 10 studied samples with each other, and the reason may be due to the great similarity in the sequence of bases that belong to the ITS of one species, and this is consistent with the study that confirmed that genetic homogeneity in the sequence of ITS genes within a species is common (Chen., et al 2007). Another study confirmed that all copies of ITS within each strain had very similar sequences (Ruiz et al., 2000)

Table 6
Similarity of ratios of studied *A. baumanni* isolates vs. source bacteria A. baumanni NZ_CP043953.1

	NZ_ CP043953.1:	AcB1	AcB2	AcB3	AcB4	AcB5	AcB6	AcB7	AcB8	AcB9	AcB10
NZ_ CP043953.1	100%	99%	97%	98%	98%	99%	98%	99%	97%	97%	97%
AcB1	99%	100%	97%	99%	99%	98%	99%	100%	97%	97%	97%
AcB2	98%	98%	100%	98%	98%	97%	98%	98%	100%	100%	100%

AcB3	99%	99%	98%	100%	99%	100%	99%	100%	98%	98%	97%
AcB4	100%	100%	99%	100%	100%	99%	100%	100%	99%	99%	98%
AcB5	100%	99%	97%	100%	99%	100%	98%	99%	97%	97%	97%
AcB6	99%	99%	98%	99%	99%	98%	100%	100%	98%	98%	98%
AcB7	100%	100%	98%	99%	99%	99%	99%	100%	98%	98%	98%
AcB8	98%	98%	100%	98%	99%	98%	98%	99%	100%	100%	100%
AcB9	98%	98%	100%	98%	99%	98%	98%	99%	100%	100%	100%
AcB10	98%	98%	100%	97%	98%	97%	98%	98%	99%	99%	100%

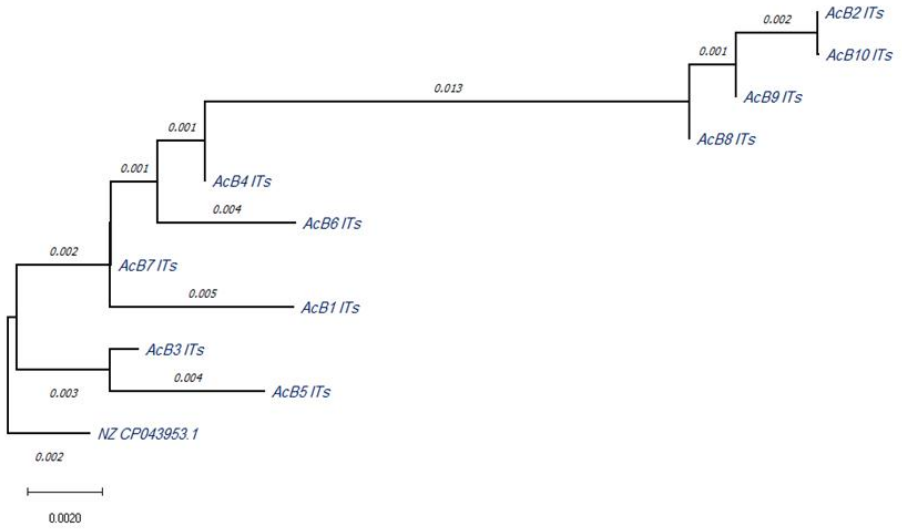

Figure 3. Genetic analysis of *A. baumannii* isolates and the reference gene using the neighbor-and-combination method. Evolutionary distances were calculated using the compound maximum probability method

In this study, genetic analysis of 10 isolates of *A.bumannii* obtained from our study was conducted and compared with the source bacteria NZ_ CP043953.1, where it was found that the closest evolutionary distance to the source bacteria is sample No. 7 and then sample No. 3 and this means that these samples have the least genetic changes in return. It was found that the furthest evolutionary distance from the source bacteria was isolate No. 2, then isolate No. 10, 9 and 8, respectively. On the other hand, it was found that the closest evolutionary distance to the source bacteria was isolate No. 3 and then isolate No. 5 as shown in Figure (1).

It is clear in this figure that isolate No. 2 is close to isolate No. 10, and they also appeared close to isolates 9 and 8. In contrast, it was found that isolate No. 2 has the farthest evolutionary distance from the source bacteria, followed by isolate No. 10, 9 and 8, and the reason may be due to the occurrence of several mutations based on ITS analysis That is due to them, as the length of the branches in the analytic tree depends on the genetic changes that occur with time. This is consistent with the study that showed that genetic variables accumulate over time slowly by mutations, where genetic changes occur mostly due to horizontal

gene transfer that allows the transfer of genes from one species to another. (Else Levin, 2021).

Phylogenetic tree of *A.bumannii*

Genetic analysis of a portion of the ITS of *A. bumannii* of the (PA) sample was carried out to determine the closest genetic similarities to A. NCBI Gene Bank If the percentage of matching is high with the isolate gene CP051862.1 isolated from as it appeared with the gene isolate CP053098.1 isolated from the United States of America followed by the gene isolate CP040080.1 isolated from India, the reason for this genetic affinity may be due to infection transmitted It has bacteria between countries because of travel or because of wars. Where bacteria were seen among wounded soldiers in the Gulf War (Chusri et al., 2019). Another study showed infection caused by *A. baumannii* during the 2003-2005 military operations in Iraq. In another study of US military service members injured in Iraq or Afghanistan (Moubareck, *et al;*2020). Resistant bacteria are also transmitted between countries as a result of international medical care(Nakazawa,*et al;*2013). It was also found that the bacteria under study are far-identical with the isolate gene FJ17655601 isolated from Taiwan, as it was far-identical with the isolate gene CP034427.1 isolated from China as shown in the figure(2).

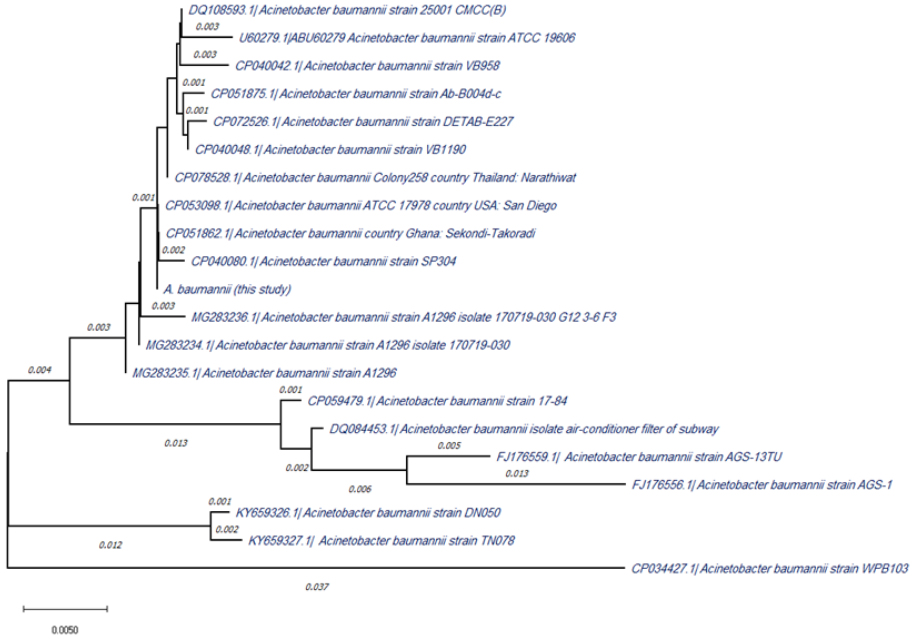

Figure 4. Genetic analysis of *A. baumanni* isolates using neighbor and linkage method. In this method, the closest reference and international representative isolates of *A. baumanni* isolates were identified from this study.

The tree is plotted at scale, with branch lengths (next to branches) in the same units as the phylogenetic distances used to infer the phylogenetic tree and the

phylogenetic distances were calculated using the compound maximum probability method.

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