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Moleculer Study of Acenitobacter Bumannii Associated with Corona Virus Disease

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***Abstract*---**In this current study, 120 samples were taken from Corona virus patients of different ages and of both sexes (males and females), who are in isolation units for Corona virus disease in hospitals in Muthanna Governorate during the period from (September to April) and were cultivated in the Chrom agar Base *Acinetobacter.* After determine the phenotypic and external characteristics, and conduct chemical tests, Then it was diagnosed by 16S-23S ribosomal DNA intergenic spacer region after conducting chemical and molecular tests, 24 out of 120 isolates were confirmed as *Acenitobacter bumannii*, Where 10 positive samples were selected for testing 16S-23S ribosomal DNA intergenic spacer region It was diagnosed as *Acenitobacter bumannii.* The gene was diagnosed PonA gene responsible for the enzyme penicillin-binding protein 1a, The gene responsible for the enzyme Beta-Lactamases PER-1 is the BlaPER1 gene, As the most important virulence factors in *Acenitobacter bumannii*, Because the two enzymes played an important role in the pathogenesis of these bacteria. And the result was (100% of the PonA gene and 40% of the blaPER1 gene).

***Keywords*---**Moleculer Study, Corona Virus Disease, Acenitobacter bumannii.

Introduction

Viral respiratory infections

Acute respiratory disease is the most frequently occurring disease in all age groups globally. The disease is mostly confined to the upper airways and is self-limiting, but a small percentage can develop lower respiratory tract infections (LRTIs), such as bronchiolitis and pneumonia. Children and the elderly are at increased risk, especially in low- and middle-income countries (LMICs). Viral agents identified included all respiratory viruses commonly circulating in the US

community as well as in the hospital environment, specifically Influenza viruses that include FLU A and FLU B and RHV/EV (rhino/enteroviruses), and HCOV. (human coronaviruses), RSV (respiratory syncytial virus), HMPV (human metapneumovirus) and (parainfluenza virus) PIV and (adenovirus) ADV (Cittoi, *et al*;2020). RSV and HCOV were the third most prevalent viruses detected in hospitalized patients (Al-Romaihi, *etal;*2019). COVID-19 is the latest pandemic with high rates of morbidity and mortality worldwide.(Lovrić, et al;2020). It is known that viral respiratory infections and subsequently COVID-19 make patients susceptible to bacterial infections, and that these symptoms have worse outcomes than infection alone. (Feldman, *etal*; 2020). The most common type of infection among ICU patients was pneumonia. Infections of the bloodstream and urinary tract have also been observed. The cultured organisms from the patients included drug-resistant *Acinetobacter baumannii* (Clancy, *et al;*2020*)*

Acinetobacter baumannii

This species was isolated for the first time by the scientist Baumann in 1968, as it can be isolated from soil and water, And the clinical samples, characterized by being negative for a dye other than forced aerobic bacilli, It has emerged in recent years as an opportunistic nurse with a distinct pathology, as it is considered one of the most important pathogens causing infections acquired from hospitals, especially the intensive care unit. (Munoz-Price *et al.*,2010). *A.baumannii* can be distinguished from the rest of the species in that it grows at a temperature of 44 °C and is non-hemolytic when growing on solid blood medium.(Dijkshoorn *et al.*, 2011). They are commonly found in a hospital setting and cause Pneumonia, meningitis, necrotizing fasciitis, sepsis, urinary tract infections, skin and/or soft tissue infections, endocarditis (Priyadharsini,*et al;*2018). The injuries that were recorded in the ranks of the US military led to the interest in this pathogen, and it even acquired the name Iraqi bacter (Shea, 2012). Clinically, it is one of the most difficult pathogens to treat, as it is the second Gram-negative pathogen after Pseudomonas in clinical isolates (Yang,*et al* ;2010).

Increasing the length of hospital stay, patients undergoing surgical operations, wounds and burns, the presence of chronic diseases such as diabetes, as well as the excessive use of antibiotics, especially broad-spectrum antibiotics, or immunosuppressive treatments increase the rate of infection and spread of *A.bumanni* (Kempf and Rolain ,2012) *A.Bumannii* has a great ability to spread and colonize the host, Adhesion ability and colonization of immunocompromised patien (Dijkshoorn, *et al*; 2007).

Materials and Methods

In this study, 120 samples were collected from coronavirus patients of both sexes (males and females) lying in the isolation unit during the period from (September to April)Their ages ranged between (18-95) years .Where samples were taken from the respiratory tract using swab sticks for patients with the Corona virus, and then the swabs were placed inside airtight tubes containing a carrier culture medium to be transferred to the laboratory.

Biochemical test

In this study, several biochemical tests were used to diagnose bacteria, such as the test for indole, oxidase and catalase and the red instance test. The bacteria were also diagnosed using a Vitek2 system.

DNA extraction and purification

All studied samples of *A. bumannii* were isolated and cultured in Luria Bertani (LB) broth. After 24 hours at 35 ◦C, Genomic DNA of studied UPEC strains was extracted by using Presto™ Mini gDNA Bacteria Kit (Geneaid company, USA). The extracted Chromosomal DNAs were used as DNA templates for all PCR based assays. Procedure of this assay was performed according to manufacturer's instructions.

PCR reactions

PCR amplification of DNA was performed by thermal cycler in final mixture volume of 25 µl. PCR Products were analyzed by 1.5% agarose gel electrophoresis and illustrated under UV trans-illuminator after staining with ethidium bromide.

Table No. (1) Diagnosis of *A. bumannii* by 16S-23S ribosomal DNA intergenic spacer region(ITS)

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 (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	°C95	min5	1
	AGAGCACTGTGCACTTAAG	PA-R	°C94	Min1	30

			°C 55	Min1	
			°C 72	Min1	
			°C 72	Min 10	1

Table (3) represents the optimal combination for PonA gene detection

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) represents the reaction cycle conditions for diagnosing PonA gene

The gene	Initiator sequence		temperature	Time	Number of cycles
PonA gene	TCCTCACCCGGTAAGTCATC	PA-F	°C 95	min5	1
	GCTCAGGCACCTATTTCTCG	PA-R	°C94	Min1	30
			°C56	Min1	
			°C72	Min1	
			°C72	Min10	1

Table (5) represents the optimal combination for BlaPER1 gene detection

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 (6) Terms of the reaction cycle for the gene Beta-Lactamases PER-1

The gene	Initiator sequence		temperature	Time	Number of cycles
BlaPER1 gene	CCAGCTGCTTTGCATGACTA	PA-F	°C 95	min5	1
	CTCTGGTCCTGTGGTGGTTT	PA-R	°C94	Min1	30
			°C57	Min1	
			°C72	Min1	
			°C72	Min10	1

Results and Discussion
Isolation and diagnosis

The study included 120 samples of corona virus patients of both sexes (males and females) who are in the isolation unit for corona virus patients During the period (from September to April), where samples were taken in the form of swabs from the upper nasopharyngeal passage, the patients' ages ranged from 18 to 95 years. And by planting it on CHROMagar *Acinetobacter* media and After conducting biochemical and molecular tests, 24 positive samples of *A.bumannii* bacteria were obtained out of 120 samples. The results of the study that were conducted in agreement with the results of the study that was conducted in Badr Eldeen (2021) who found 25l120 *Acinetobacter* bacteria by culture in CHROMagar TM *Acinetobacter* medium. In this study, information was collected from Corona virus patients (age, gender, does the patient have chronic diseases, is the infection for the first time or not?, is the patient subject to a vaccine?, what is the type of vaccine?

Table (7) Distribution of the number of corona patients and samples by age

Age group	The number of corona patients	%	Number of positive results for *Acenitobacter bumannii*	%
15 – 25	7	%5.3	1	%4.16
26 – 35	4	%3.3	--	%0
36 – 45	9	%7.5	1	%4.16
46 – 55	16	%13.3	2	%8.33
56 – 65	20	%16.6	4	%16.66
66 – 75	32	%26.6	4	%16.66
76 – 85	19	%15.8	8	%33.33
86 – 95	13	%10.8	4	%16.66
Total	120	%100	24	%100

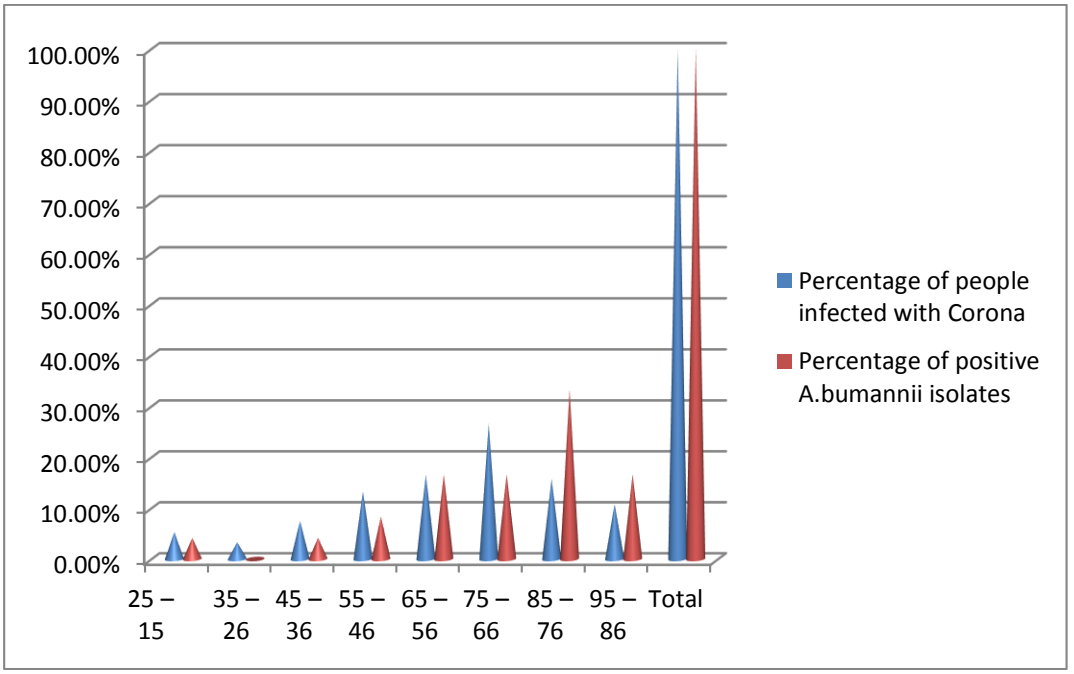

Figure (1-1) shows the percentages of corona patients and the percentages of samples positive for *A. bumannii.*

It was found that the spread of corona disease among the ages of 56 years and above was the most, as shown in Table No. (7), where we note in the table that the ages (56-65) had a disease rate of 16.6%, and that the ages (66-75) had a percentage The disease is 26.6%, and the ages (76-85) have a disease rate of 15.8%, which confirms that the Corona virus has more impact on the elderly. These results were consistent with the study (Mueller, et al; 2020), where it confirmed that the severity and outcomes of Coronavirus Disease 2019 (COVID-19) depend to a large extent on the age of the patient. Adults over 65 years of age account for 80% of hospital admissions and have a 23 times greater risk of death than those under 65 years of age. We also note in our study that the number of positive isolates of *A. bumannii* bacteria increases at the age of 56 and above, and this is due to the weakness of the immune system of this group, which encourages pathogens to attack the body(CDC, 2001).

Table (8) Microscopic and Cultivational Characteristics and Biochemical Tests of *A. baumannii*

Test type	The result
Lactose fermentation	-
Haemolysis Production	-
Growth in 44C°	+
Gram Stain	-
Oxidase	-

Catalase	+
Urease	Variable
Indol	-
Methyl red	-
Voges_ proskaure	-
Simmon citrate	+
Motility	-

* + : test positive *- : test Negative

10were selected samples positive for the 16S-23S ribosomal DNA intergenic spacer region and diagnosed as *A.bumannii* Which underwent a test for the gene responsible for the enzyme Penicillin-Binding Protein 1a (PonA gene) Because of the importance of the enzyme Penicillin-Binding Protein 1a as an important virulence factor for *A. bumannii* pathogenesis. The enzyme PBP1a plays a key role in septum formation during the cell division cycle and its modification is essential for the development of high-level resistance to penicillin and Contreras-Martel,*et al;*2006). (cephalosporin

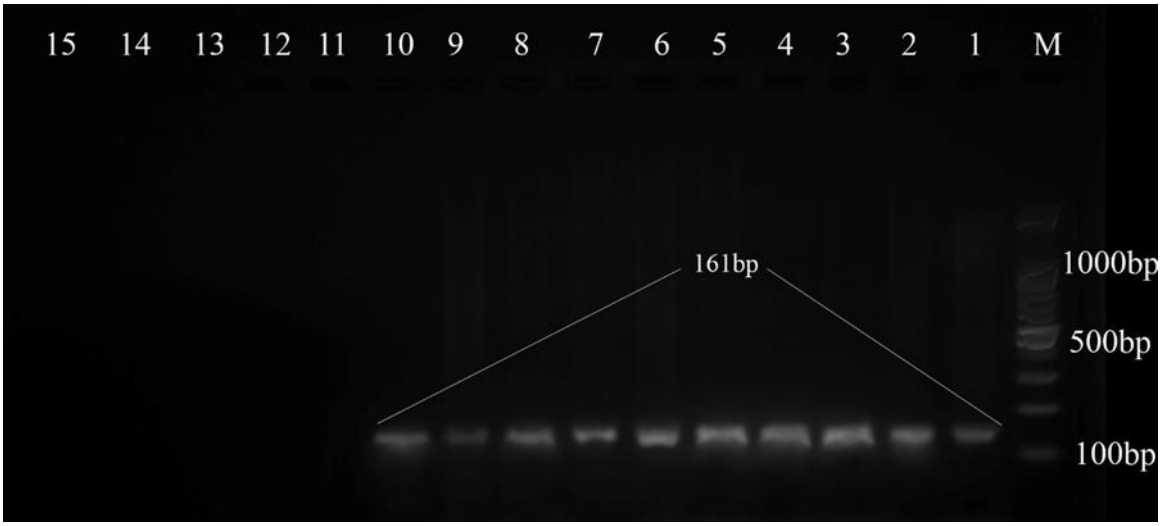

Fig. (1-2): Agarose gel electrophoresis of PCR products obtained by using penicillin-binding protein PBP1a-specific primer. lanes 1-10 represent the identified PBP1a gene products with 161bp, Lane M represent 100bp DNA ladder.

Table (9): Identification of PBP1a gene of *Acinetobacter baumannii* in all studied samples

Results	N	Percentage	P value
Positive	10	(100)	<0.0001*
Negative	0	(0)	
Total	10	100%	

* represent a significant difference at p<0.05.

In addition to testing the gene responsible for the enzyme Beta- Lactamases PER-1 (BlaPER1 gene), Beta-lactamases are enzymes that catalyze the hydrolysis of

beta-lactam antibiotics and can be divided into four classes based on sequence motifs and differences in the hydrolytic mechanism (Tooke,*et al;* 2019) . They are class A, B, C, D, E, and beta-lactamases PER-1 belongs to class A. Class A beta-lactamases mediate resistance to penicillin, cephalosporins, monobactams and carbapenems
Ghafourian, *et al;*2015).
This enzyme is encoded by the PER-1 gene, but this gene is related to cell adhesion. Nine PER-1-producing strains are bound to Caco2 cell lines, while all PER-1-negative strains are negative for cell adhesion..*et al*;2016),Koenigs(

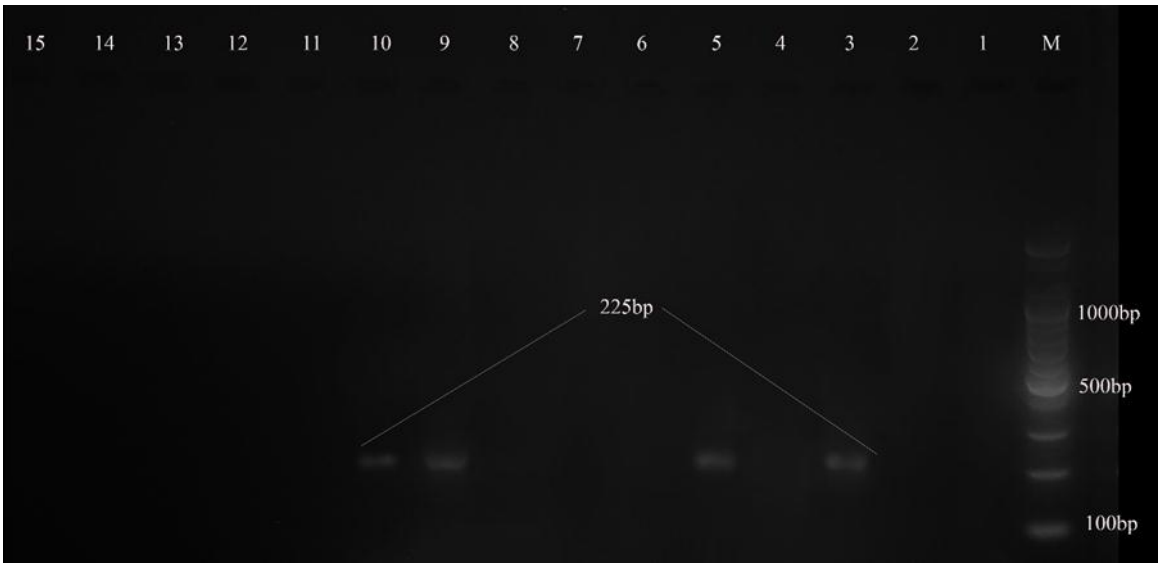

Fig. (1-3): Agarose gel electrophoresis of PCR products obtained by using blaPER1-specific primer. lanes 3,5,9 and 10 represent the identified blaPER1gene products with 225bp, Lane M represent 100bp DNA ladder.

Table (10): Identification of blaPER1gene of *Acinetobacter baumannii* in all studied samples

Results	N	Percentage	P value
Positive	4	(40)	<0.0001*
Negative	6	(60)	
Total	20	100%	

* represent a significant difference at p<0.05.

The result was that all samples contained bacteria belonging to the genus *A. bumannii* 100% of the PonA gene, and 40% of the blaPER1 gene, and this confirms the importance of these bacteria containing both enzymes.

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