

RESEARCH / INVESTIGACIÓN

Determination of the prevalence of *bla_{oxa}-like* gene and *ISAb_a1* elements among extensive-drug resistant (XDR) *Acinetobacter baumannii* isolates

Salah Mohsin¹, Wasan Abdul-Elah Bakir¹, Majeed Arsheed²

DOI. 10.21931/RB/2021.06.04.25

Abstract: The capacity of Multi-drug resistant (MDR) *Acinetobacter baumannii* to survive in any state of affairs concerning the gaining of various gene types of virulence and antimicrobial agent resistance are the main anxiety in the hospital's environments. So, it is very crucial to determine the prevalence of insertion sequences in *A. baumannii* in the hospitals. Detecting the *bla_{oxa}-51* gene through the polymerase chain reaction (PCR) was performed to confirm *Acinetobacter baumannii* and the search for *ISAb_a1* element. Between October 2020 and February 2021, 540 distinct clinical specimens were gathered from five hospitals in Baghdad. Thirty-eight *A. baumannii* isolates were obtained from various clinical specimens. The isolates were initially identified phenotypically using standard microbiological techniques and by the Vitek2 compact automated machine. Isolates of *A. baumannii* were identified genotypically by amplification of the *bla_{oxa}-51-like* gene. Antimicrobials are studied by Kirby-Bauer (disc diffusion) technique on Muller-Hinton agar as specified by the recent clinical and laboratory standard institute (CLSI) guidelines (2020). The actual results of the current study indicated that from total isolated (38) *A. baumannii* isolates, 23 isolates (61%) were resistant to meropenem and 25 isolates (66%) were resistant to imipenem. The *bla_{oxa}-51* gene was identified in all strains examined, *ISAb_a1* was also present in all *A. baumannii* isolates. *ISAb_a1* has a high predominance between drug-resistant *A. baumannii*. Identifying these parameters can assist in the control of infection and decreasing the microorganism's prevalence rate.

Key words: Insertion sequence, The *bla_{oxa}-51-like* gene, *Acinetobacter baumannii*.

Introduction

An opportunistic infectious agent, *A. baumannii* encompasses a high rate of occurrence among immunocompromised people, significantly those that have experienced long (more than ninety days) hospital residence¹. Commonly related to aquatic environments². It's been recognized as a "red alert" human infectious agent in recent years, causing concern among medical professionals, owing to its extensive spectrum of antibiotic resistance³.

The development of multidrug-resistant (MDR) pathogens has more and more become a cause for profound importance concerning each healthcare facility and community-acquired infections⁴. According to the World Health Organization (WHO), Antimicrobial resistance has recently been recognized in concert as one of the 3 most vital issues facing human health⁵. The therapeutic selections are restricted, typically leading to unsuitable medical care and resulting negative consequences on patient⁶.

The primary antibiotic resistance mechanisms are enzymatic (production of β -lactamases and enzymatic modification of aminoglycosides) and non-enzymatic (changing membrane permeability, activating efflux pumps) and altering of the target site⁷.

Insertion sequences (IS) are among the most basic mobile genetic elements (MGEs) and are found across the animal kingdom. To present, more than 4500 IS from 29 families have been discovered^{8,9}. In *Acinetobacter* spp., more than thirty different types of Insertion Sequences have been discovered, suggesting that ISs had a significant role in developing this species and contributing to the multidrug-resistant phenotype shown in this genus¹⁰.

Insertion elements have two distinct characteristics: short transposable elements (up to 2500bp) and only code for proteins involved in transposition¹¹.

In *A. baumannii*, *ISAb_a1* has been found in conjunction

with numerous antibiotic resistance genes¹². *ISAb_a1* has been shown to function in the expression of the *Bla* ampC gene, the antibiotic resistance gene of *A. baumannii*, which encodes the naturally occurring cephalosporins enzyme, and the *blaOXA-23* gene, which encodes a carbapenem-degrading oxacillinase. Nevertheless, it could also act in the case of other resistance genes^{13,14}. A composite transposon (defined as Tn2006) formed by two copies of *ISAb_a1* bracketing this β -lactamase gene, responsible for the movement of *blaOXA-23*¹⁵.

ISAb_a1 has been found to regulate the expression of the *bla* ampC gene, which encodes the naturally occurring cephalosporins enzyme, and the *blaOXA-23* gene, which encodes a carbapenem-hydrolyzing oxacillinase enzyme. However, it may also play a role in the development of additional resistance genes. *ISAb_a1* has been demonstrated to produce carbapenem resistance in *A. baumannii* by causing overexpression of the naturally existing *bla_{oxa}-51-like* gene¹⁶.

The expression of the *blaOXA-23* gene has been linked to *ISAb_a1* and *ISAb_a4*^{15,17}. The expression of the *blaOXA-58* gene has been related to the insertion sequences *ISAb_a1*, *ISAb_a2*, *ISAb_a3*, and *IS18*¹⁸. Therefore, determining the prevalence of insertion sequences genes in *A. baumannii* in hospitals is very crucial.

Materials

Specimens' collection

Between October 2020 and February 2021, 540 distinct clinical specimens were collected from five hospitals in Baghdad, including The Burns Hospital, Gazi Al-Hariri Hospital, Baghdad Teaching Hospital, Welfare Teaching Hospital in the Medical City, and Al-Yarmouk teaching hospital. Collected

¹ Department of Microbiology, College of Medicine, Mustansiriyah University, Baghdad, Iraq.

² Gene bank Department, Forensic DNA for research and training Centre, Al-nahrain University, Baghdad, Iraq.

specimens were sputum, blood, fluids such as (cerebrospinal, pleural, and peritoneal), urine, and swabs.

Isolation of Bacteria

In the laboratory (Teaching Laboratories/Medical City, Baghdad) under aseptic conditions, the collected specimens were cultured directly on MacConkey agar and blood agar, incubated for 24 hrs. at 37°C. The colonies were non-hemolytic opaque creamy on blood agar, while on MacConkey agar were non-lactose fermenting colonies. For obtaining pure, well-isolated colonies, they were subcultured on another MacConkey agar plate and incubated for another 24 hrs. at 37°C¹⁹.

Bacterial Identification

Microscopical examination

One isolated colony was transferred to a microscopic slide, fixed then stained with Gram stain (GS). Gram reaction, cell shape, and arrangement were recorded. The results were compared with Brooks *et al.* (2013)²⁰.

Biochemical tests

To phenotypically identify the isolates as *A. baumannii*, biochemical tests such as the ability to grow at 42°C, culture on selective medium, negative for oxidase test, absence of lactose fermentation, and others were utilized.

Identification of *Acinetobacter baumannii* using VITEK® 2 system.

As stated by the manufacturer's instructions, identification cards of Gram-Negative Bacteria (ID-GNB) were used on the VITEK® 2 system to recognize isolates at the species level. The bacterial isolates were inoculated at 37°C on MacConkey agar plates and, after incubated overnight, taken a single colony then suspended. In 0.45 % sodium chloride, the turbidity measurement for the bacterial suspension to meet the McFarland (0.5) standards, The (Gram-Negative Vitek 2 Identity card) then was manually placed into the Vitek-2 system, along with the bacterial suspension tub, the software also prepared

according to (BioMerieux, France) the manufacturer's instructions²¹.

Identification of *A.baumannii* by PCR

Using a primer specific for *bla OXA51-like* genes, PCR was utilized to amplify *bla OXA51-like* genes used for *A. baumannii* isolate recognition.

Test of antimicrobial susceptibility profile

According to the latest clinical and laboratory standard institute (CLSI) criteria (2020), the isolates were tested for antimicrobial susceptibility to 18 antimicrobial agents using the Kirby-Bauer disc diffusion technique on Muller-Hinton agar (Oxoid /England)²². The antibiotic discs (Mast Group /UK) used throughout the study for *A.baumannii* isolates are Piperacillin-Tazobactam (100/10 µg/disc), Ampicillin-sulbactam (13/10 µg/disc), Ticarcillin-Clavulanate (55/13 µg/disc), Cefepime (33 µg/disc), Cefotaxime (33 µg/disc), Ceftazidime (33 µg/disc), Ceftriaxone (33 µg/disc), Imipenem (13 µg/disc), Meropenem (13 µg/disc), Colistin sulphate (25 µg/disc), Tobramycin (13 µg/disc), Gentamicin (13 µg/disc), Amikacin (33 µg/disc), Doxycycline (30 µg/disc), Tetracycline (30 µg/disc), Ciprofloxacin (5µg/disc), Levofloxacin (5µg/disc) and Trimethoprim-Sulphamethoxazole (1.25/ 23.55 µg/disc).

Genomic DNA extraction from bacterial isolates

Extraction of bacterial DNA from isolates under study using a commercial extraction system (ZR Fungal/Bacterial DNA Miniprep Kit) designed to isolate DNA from Gram-negative bacteria according to the manufacturer's instructions. For each reaction, a totally of 4 µl of extracted DNA was used.

Molecular recognition of *BlaOXA-51-like* gene and Insertion sequence elements

To detect XDR *A.baumannii* isolates, PCR was used to detect the *Bla-OXA-51-like* gene and *ISAbal* elements. Primer sequences for each gene reported above are listed in table (1). These primers (Macrogen, South Korea) were received in a lyophilized state, dissolved in sterile deionized distilled water

Primer	The sequence of primers (5' ---3')	Product size	Accession number
OXA51	CTTTTGGCTAAATGGAAGCG	434	CP081137.1
OXA51	CGGGTGTCTTAGTTATCCAAC		
<i>ISAbal</i> F	CACGAATGCAGAAGTTG	549	CP029569.1
<i>ISAbal</i>	CGACGAATACTATGACAC		

Table 1. Sequences of primers used throughout the study.

Steps	OXA-51	<i>ISAbal</i>	Repeats
Activation	94°C/5min	94°C/5min	1 cycle
Denaturation	94°C/45s	94°C/45s	40 cycles
Annealing	56°C	50°C	
Extension	72°C/45s	72°C/45s	
Final extension	72°C/7min	72°C/7min	-

Table 2. Programs were used in the PCR for *OXA-51-like* gene and *ISAbal* element.

to a final concentration of 100 picomole/ μ l, and kept in a deep freezer until use, as advised by the vendor.

The PCR amplification procedure for the genetic level to detecting genes under study by follows steps: Final volume for PCR mixture was 25 μ l (12.5 of Green Master Mix 2x, 4 μ l extracted template DNA, 1.5 μ l from each forward and reverse primer, 5.5 μ l nuclease-free water were added in 0.2 ml PCR Eppendorf tubes, mixed for a short time via vortex then been loaded to Veriti™ 96-Well (applied biosystems) Thermal Cycler. The program used for each monoplex PCR reaction was set according to each primer. The best annealing temperature was chosen after the gradient runs through the optimization process of each oligonucleotide primer.

For amplification of the *Bla-OXA-51-like* gene, the DNA thermal cycler device Veriti™ 96-Well (applied biosystems) was programmed in the following amplification conditions: Following a 5-minute activation at 94°C, 40 cycles of 45 seconds at 94°C (denaturation), 56°C (annealing), and 45 seconds at 72°C (extension) were conducted. In contrary to other genes, the ISAbal annealing temperature was 50°C. The last cycle was followed by 7 minutes at 72°C (Table 2). Amplified PCR products were examined on 1.5% agarose gel at an electrical current of 7 volt/cm² in 1X TBE buffer with added Red safe dye (INTRON) has been exposed till the tincture had reached the other side of the gel. The SiZer™-1000 Plus DNA Marker and SiZer™-100 DNA Marker (Intron / Korea) were used as markers during PCR products electrophoresis. After that, the agarose gel was removed from the tank and visualized by a UV transilluminator documentation system (Cleaver scientific /UK) at 336 nm, then photographed using a digital camera.

Sequencing was carried out by Macrogen DNA Sequencing (Seoul, Korea) using 3730xl DNA Analyzer (Applied Biosystems™, Foster City, CA). Two samples from ISAbal PCR products with forwarding primer (17 pmol/ μ l) for each gene were selected and sent to sequencing. Raw reads generated in this study were trimmed or filtered to remove low-quality sequences using (SnapGene software). Once sequencing reads had been obtained, the data analysis process was started, the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI) was used to analyze DNA sequences and do similarity searches.

Results

540 clinical specimens were collected between October 2020 and February 2021 from some hospitals in Baghdad, including Baghdad medical city and Al-Yarmouk hospitals. Thirty-eight bacterial isolates were obtained, which differentiated to *A.baumannii*. Following conventional identification techniques, antimicrobial sensitivity testing was carried out by examining the findings in (Figure 1), revealing a high degree of resistance of *A.baumannii* clinical isolates to the majority of the antimicrobial agents under test.

In this study, antibiotic resistance profile of *Acinetobacter baumannii* for 18 antibiotics as following: Trimethoprime/sulphamethoxazole 32 (84%), Ciprofloxacin 27 (71%), Piperacillin/tazobactam 26(68%), Ticarcillin/clavulanate 26 (68%),Cefazidime 26(68%), Ceftriaxone26(68%), Cefotaxime 25 (66%), Imipenem 25(66%), Doxycycline 26(68%), Levofloxacin 25 (66%),Cefepime 24(63%),Tetracycline 24(63%), Meropenem 23(61%), Gentamicin 22 (58%), Tobramycin 15(39%), Amikacin 17(45%), Ampicillin/sulbactam 12 (32%), Colistin 0(0%). The exact and important results of current study indicated that from total isolated (38) *A.baumannii* isolates, 23 isolates (61%)

and 25 isolates (66%) were resistant to meropenem and imipenem respectively.

All the *A. baumannii* isolates were positive for *bla_{oxa-51-like}* genes and ISAbal genes. Figures 1 and 2 demonstrate agarose gel electrophoresis of PCR products of *bla_{oxa-51-like}* genes and ISAbal elements, respectively.

The sequence was analyzed by BLAST software at NCBI. Figure (4) shows the alignment result with the USA: San Diego isolates (Accession number CP053098.1). The sequence was found to share 99% nucleotide homology with the reference isolates. Tables 3 and 4 show nucleotide changes and Features of ISAbal(Forward) from *Acinetobacter baumannii* (X9 isolate) with *Acinetobacter baumannii* ATCC 17978 chromosome from the USA: San Diego.

The sequence was analyzed by BLAST software at NCBI. Figure (4) shows the alignment result with USA: San Diego isolates (Accession number CP050388.1). The sequence was found to share 99% nucleotide homology with the reference isolates. Tables 5 and 6 show the nucleotide changes and features of ISAbal(Forward) from *Acinetobacter baumannii* (X10 isolate) with *Acinetobacter baumannii* strain VB473 chromosome from India.

Discussion

A. baumannii has developed as a well-established nosocomial pathogen with a high level of antibiotic resistance. Various medical facilities frequently report extensively drug-resistant, and pan drug-resistant isolates²³. By 2007, up to 70% of isolates in specific locations had evolved multidrug resistance, particularly resistance to carbapenems, which were formerly thought to be the gold standard for treating MDR *A. baumannii* infections²⁴.

The antibiotic of choice for treating *A. baumannii* is carbapenems. Due to rising resistance rates, *A. baumannii* infections are becoming increasingly ineffectual. Resistance to the newer antibiotic tigecycline is also quickly developing. Colistin, a previously abandoned antibiotic, is now used as a last option, yet resistance to this medication is increasing at an alarming pace throughout the world²³.

Aside from its proclivity for the critically ill in intensive care units, *A. baumannii* has lately been linked to a slew of infectious diseases among military troops injured in the Iraq and Afghanistan wars⁴.

Resistance of Carbapenem is frequently connected to the making of oxacillinase enzymes. Metallo β -lactamases (MBL), on the other hand, can cause carbapenem resistance in *A. baumannii*²⁵.

The actual results of the current study indicated that of 38 *A.baumannii* isolates, 23 isolates (61%) and 25 isolates (66%) were resistant to meropenem and imipenem, respectively. The antibiotic resistance results of imipenem and meropenem are less than that found by al Al-Saadi (2018)²⁶. From 162 *A.baumannii* isolates, 112 isolates (88.19%) and 107 (84.25%) were resistant to meropenem and imipenem, respectively. Prior investigators in Iran found that the resistance rates to imipenem and meropenem were (95.23%) and (98.09%) respectively²⁷, indicating that these bacteria have a wide range of resistance mechanisms. This would imply significant risks among hospitalized patients, mainly where this antibiotic class was previously considered the standard therapy for *A.baumannii* infections²⁸.

The recognition of the *Bla OXA-51-like* gene can be utilized to identify *A.baumannii* reliably and straightforwardly^{29,30}.

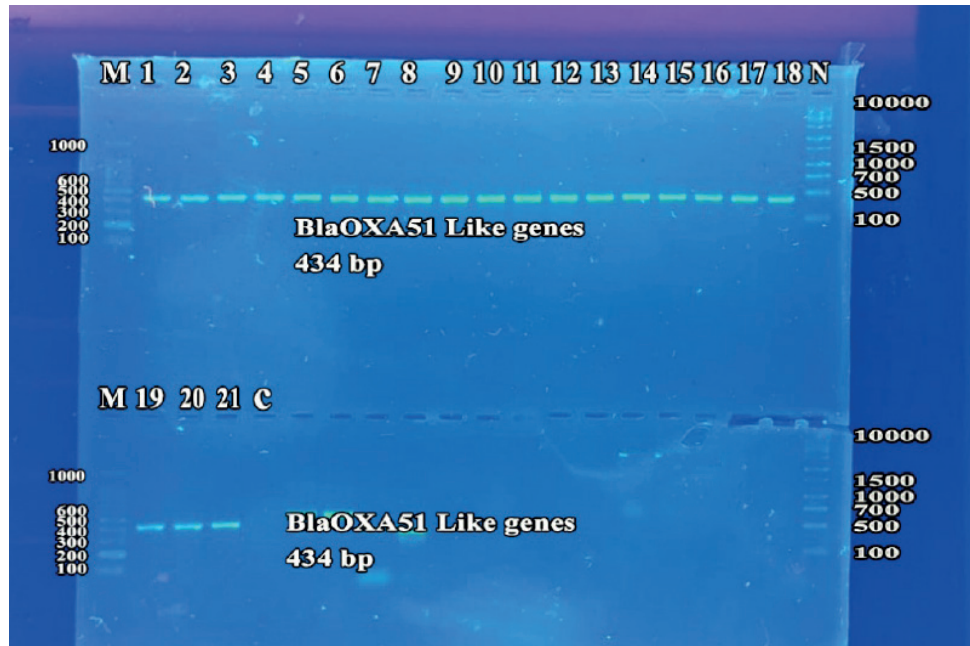


Figure 1. Detection of *bla*OXA51 like gene by monoplex PCR for *A.baumannii* isolates. lanes 1-21, XDR *A.baumannii*; Lane C, Negative control. Lane M, 100 bp DNA marker. Lane N, 1000 bp plus DNA marker. Detection was done on agarose gel (1.5%) at 5 Volt/cm for 1.5 hours, stained by Red Safe dye, and imagined on a UV transilluminator documentation system.

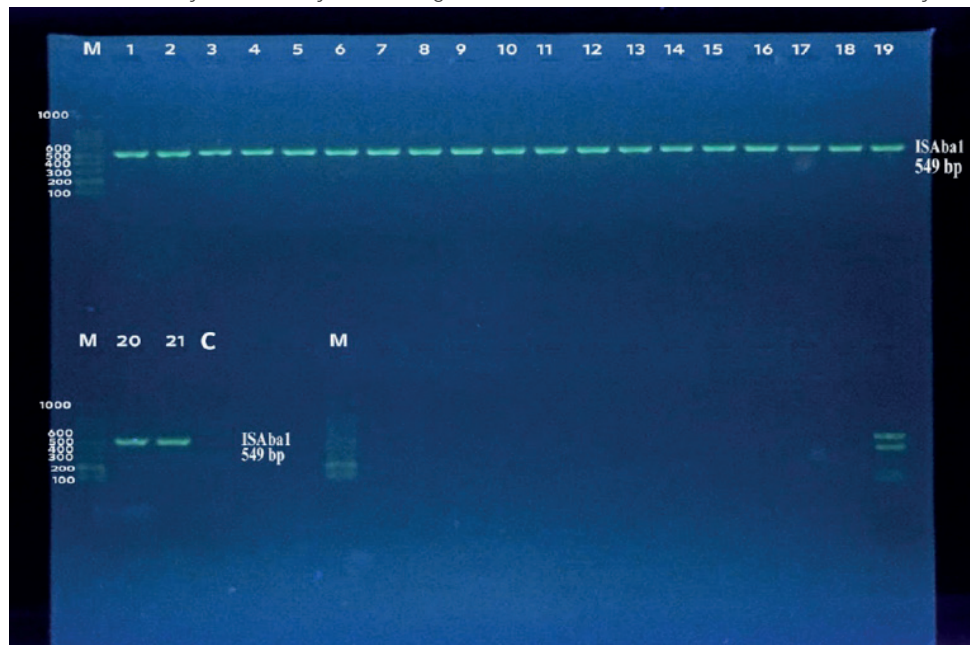


Figure 2. Detection of IS*Aba*1 gene by monoplex PCR of isolates. Lanes 1-21: XDR *A.baumannii* isolates. Lane C: Negative control. Lane M: 100 bp DNA marker. Detection was done on agarose gel (1.5%) at 5 Volt/cm for 1.5 hours, stained by Red Safe dye, and imagined on a UV transilluminator documentation system.

Furthermore, because this gene was controlled by insertion sequences such as IS*Aba*1, the presence of intrinsic chromosomally placed genes of the *bla* OXA-51-like gene did not correlate with the amount of carbapenem resistance of *A.baumannii* isolates³¹.

Additionally, all isolates of *A.baumannii* carried the *Bla* OXA-51-like gene and attributed the imipenem resistance state to the presence of IS*Aba*1 upstream of the *bla_{oxa}-51-like* gene serves as a promoter for gene expression as one of these isolates' resistance methods^{32,33}.

From 21 XDR *A. baumannii* isolates all have *bla_{oxa}-51* like genes. This corroborated those of previous local investigations^{26,34,35}.

A study in Egypt revealed that genes encoding *bla_{oxa}-51* (belonging to class D carbapenemases) were found in 100% of the studied isolates³⁶. According to research by Bahador et al., all 62 CRAB isolates tested positive for *bla_{oxa}-51-like* genes³⁷.

The present data could affirm that all *A.baumannii* isolates were positive for the IS*Aba*1 gene. It was found that the prevalence of IS*Aba*1 was 100%^{27,36}. IS*Aba*1 was the most common insertion element (90.6%)³⁸. The prevalence of IS*Aba*1 is equal to that seen in 59 Spanish isolates (93.2%)³⁹. While Taiwan (36%)⁴⁰ and India (33%)⁴¹ have lower prevalence rates than the rest of the world. The presence of various insertion sequences in *A. baumannii* makes it resistant to carbapenems⁴².

These insertion sequences are found near genes that code

Gene: <i>ISAbal</i> (Forward) from <i>A.baumannii</i> (X9 isolate)						
No.	Type of substitution	Location	Nucleotide	Sequence ID with compare	Source	Identities
1	Deletion	52	-A	CP053098.1	<i>Acinetobacter baumannii</i>	99%

Table 3. Nucleotide changes of *ISAbal*(Forward) from *Acinetobacter baumannii* (X9 isolate) with *Acinetobacter baumannii* ATCC 17978 chromosome from the USA: San Diego.

Feature	Studied isolate	Reference isolate
Molecule Type	Genomic DNA	Genomic DNA
Isolation Source	Swab	Fatal meningitis
Host	Homo Sapiens	Homo Sapiens
Country	Iraq, Baghdad	USA: San Diego

Table 4. *ISAbal*(Forward) features from *Acinetobacter baumannii* (X9 isolate) with *Acinetobacter baumannii* ATCC 17978 chromosome from the USA: San Diego.

Gene: <i>ISAbal</i> (Forward) from <i>A.baumannii</i> (X10 isolate)						
No.	Type of substitution	Location	Nucleotide	Sequence ID with compare	Source	Identities
1	Deletion	83	-A	CP050388.1	<i>Acinetobacter baumannii</i>	99%
	Deletion	85	-A			

Table 5. Nucleotide changes of *ISAbal*(Forward) from *Acinetobacter baumannii* (X10 isolate) with *Acinetobacter baumannii* strain VB473 chromosome from India.

Feature	Studied Isolate	Reference Isolate
Molecule Type	Genomic DNA	Genomic DNA
Isolation Source	Swab	Sputum
Host	Homo Sapiens	Homo Sapiens
Country	Iraq, Baghdad	India

Table 6. Features of *ISAbal*(Forward) from *Acinetobacter baumannii* (X10 isolate) with *Acinetobacter baumannii* strain VB473 chromosome from India.

for several OXA-type carbapenemases and are implicated in their overexpression¹³.

Conclusions

In conclusion, *A. baumannii* is a significant pathogen in several nations. According to the findings of this study, it has a high resistance rate against most antibiotics, threatening in patients as a red alarm bacterium in hospitals, producing a high rate of death and morbidity due to its numerous mechanisms of resistance and the fact that it is not or only rarely treated with conventional antibiotics.

This bacterium can cause dangerous and long-term infections, especially in youngsters and people with immunological deficiencies. Our research focused on specific mobile components transported between species to change the antimicrobial pattern and enhance antimicrobial resistance. *ISAbal* has a high prevalence among extreme drug-resistant *A. baumannii* isolated from several Baghdad hospitals. Identifying these parameters can aid in controlling infection and reducing the microorganism's prevalence rate.

Bibliographic references

- Montefour K, Frieden J, Hurst S, Helmich C, Headley D, Martin M, et al. *Acinetobacter baumannii*: an emerging multidrug-resistant pathogen in critical care. *Crit Care Nurse*. 2008;28(1):15–25.
- Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL. Identification of *Acinetobacter baumannii* by detection of the bla OXA-51-like carbapenemase gene intrinsic to this species. *J Clin Microbiol*. 2006;44(8):2974–6.
- Cerqueira GM, Peleg AY. Insights into *Acinetobacter baumannii* pathogenicity. *IUBMB Life*. 2011;63:1055–60. doi: 10.1002/iub.533.
- Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin. Microbiol*. 2008; 21(3): 538–582.
- Bassetti M, Ginocchio F, Mikulska M. New treatment options against gram-negative organisms. *Annu Updat Intensive Care Emerg Med* 2011. 2011;501–15.
- Rice LB: Federal funding for the study of antimicrobial resistance in nosocomial pathogens: No ESKAPE. *J Infect Dis* 2008, 197: 1079–1081.
- Vrancianu CO, Gheorghe I, Czobor IB, Chifiriuc MC. Antibiotic Resistance Profiles, Molecular Mechanisms and Innovative Treatment Strategies of *Acinetobacter baumannii*. *Microorganisms*. 2020;8(6):935. Published 2020 21 June. doi:10.3390/microorganisms8060935

Acinetobacter baumannii ATCC 17978 chromosome, complete genome Sequence
 ID: CP053098.1 Length: 4005343 Number of Matches: 1
 Range 1: 3737775 to 3738256

Score	Expect	Identities	Gaps	Strand
883 bits(478)	0.0	481/482(99%)	1/482(0%)	Plus/Minus
Query 2	CTCTGTCTGCGAACACATTCCACAATACGGTCTTTACCAAAAATGGCTATAA-GCGTTGAA	60		
Sbjct 3738256	CTCTGTCTGCGAACACATTCCACAATACGGTCTTTACCAAAAATGGCTATAAAGCGTTGAA	3738197		
Query 61	TCAAAGCAATACGCTCTTTTCGTATCTGAATTTCCACGTTTATTAAGCAATGTCCAAAGGA	120		
Sbjct 3738196	TCAAAGCAATACGCTCTTTTCGTATCTGAATTTCCACGTTTATTAAGCAATGTCCAAAGGA	3738137		
Query 121	TAGGTATCGCTATTCCACGATAAACGATTGCGAGCATCAGGATATTAATATTTTCGTTTTTC	180		
Sbjct 3738136	TAGGTATCGCTATTCCACGATAAACGATTGCGAGCATCAGGATATTAATATTTTCGTTTTTC	3738077		
Query 181	CCCATTTCCAATTGGTTCTATCTAAAGTCAGTTGCACTTGGTGAATGAAAACATATTGA	240		
Sbjct 3738076	CCCATTTCCAATTGGTTCTATCTAAAGTCAGTTGCACTTGGTGAATGAAAACATATTGA	3738017		
Query 241	AAATCAACTGAGAAATTTGACGATAATCAAAATACTGACCTGCAAAGAAGCGCTGCATAC	300		
Sbjct 3738016	AAATCAACTGAGAAATTTGACGATAATCAAAATACTGACCTGCAAAGAAGCGCTGCATAC	3737957		
Query 301	GTCGATAAAATGATTGTGGTAAGCACTTGATGGGCAAGGCTTTAGATGCAGAAGAAAGAT	360		
Sbjct 3737956	GTCGATAAAATGATTGTGGTAAGCACTTGATGGGCAAGGCTTTAGATGCAGAAGAAAGAT	3737897		
Query 361	TACATGTTTGCTTTAAAATAATCAACAAGCATGATGAGCGCAAAGCACTTTAAATGTGACT	420		
Sbjct 3737896	TACATGTTTGCTTTAAAATAATCAACAAGCATGATGAGCGCAAAGCACTTTAAATGTGACT	3737837		
Query 421	TGTTCCATTTTAGATATTTGTTTAAAGATAAGATATAAECTCATTGAGATGTGTCATAGTAT	480		
Sbjct 3737836	TGTTCCATTTTAGATATTTGTTTAAAGATAAGATATAAECTCATTGAGATGTGTCATAGTAT	3737777		
Query 481	TC 482			
	II			

Figure 3. Sequence alignment of IS*Aba*1(Forward) of *A.baumannii*(X9 isolate) with *Acinetobacter baumannii* ATCC 17978 chromosome from USA: San Diego.

- Siguiet P, Filée J, Chandler M. Insertion sequences in prokaryotic genomes. *Curr Opin Microbiol.* 2006;9(5):526–31.
- Siguiet P, Gourbeyre E, Chandler M. Bacterial insertion sequences: their genomic impact and diversity. *FEMS Microbiol Rev.* 2014;38(5):865–91.
- Montaña S, Almuzara M, Pennini M, et al. IS CR2 and IS 26: two insertion sequences highly dispersed among *Acinetobacter* spp. clinical strains. *J Bacteriol Mycol Open Access.* 2017;4(1):33-36.
- Campbell N A, and J B. Reece. *Biology* (6th ed.) Benjamin Cummings, San Francisco. 2002; pp. 345-346
- Naas T, Namdari F, Réglier-Poupet H, Poyart C, Nordmann P. Panresistant extended-spectrum β -lactamase SHV-5-producing *Acinetobacter baumannii* from New York City. *J Antimicrob Chemother.* 2007;60(5):1174–6.
- Corvec S, Caroff N, Espaze E, Giraudeau C, Drugeon H, Reynaud A. AmpC cephalosporinase hyperproduction in *Acinetobacter baumannii* clinical strains. *J Antimicrob Chemother.* 2003; 52:629–635
- Héritier C, L. Poirel, P. Nordmann. Cephalosporinase over-expression resulting from insertion of IS*Aba*1 in *Acinetobacter baumannii*. *Clin. Microbiol. Infect.* 2006;12:123-130.
- Corvec S, Poirel L, Naas T, Drugeon H, Nordmann P. Genetics and expression of the carbapenem-hydrolyzing oxacillinase gene bla OXA-23 in *Acinetobacter baumannii*. *Antimicrob Agents Chemother.* 2007;51(4):1530–3.
- Turton J F ,Woodford N , Glover J , Yarde S , Kaufmann M E ,Pitt T L. Identification of *Acinetobacter baumannii* by detection of the blaOXA 51-like carbapenemase gene intrinsic to this species. *J. Clin. Microbiol.* 2006a; 44(8): 2974-2976.
- Bogaerts P, G. Cuzon, T. Naas, C. Bauraing, A. Deplano, B. Lisoir, P. Nordmann, and Y. Glupczynski. Carbapenem-resistant *Acinetobacter baumannii* isolates expressing the blaOXA-23 gene associated with IS*Aba*4 in Belgium. *Antimicrob Agents Chemother.* 2008; 52: 4205-4206.
- Poirel L, Nordmann P. Genetic structures at the origin of acquisition and expression of the carbapenem-hydrolyzing oxacillinase gene bla OXA-58 in *Acinetobacter baumannii*. *Antimicrob Agents Chemother.* 2006;50(4):1442–8
- Forbes BA, Sahn DF, Weissfeld AS. *Diagnostic microbiology.* Mosby St Louis; 2007.

Acinetobacter baumannii strain VB473 chromosome, complete genome
 Sequence ID: CP050388.1 Length: 3948250 Number of Matches: 27
 Range 1: 164726 to 165214

Score	Expect	Identities	Gaps	Strand
891 bits(482)	0.0	487/489(99%)	2/489(0%)	Plus/Plus
Query 2		GATAAACTCTCTGTCTGCGAACACATTCACAATACGGTCTTTACCAAAAATGGCTATAAA		61
Sbjct 164726		GATAAACTCTCTGTCTGCGAACACATTCACAATACGGTCTTTACCAAAAATGGCTATAAA		164785
Query 62		GCGTTGAATCAAAGCAATACGCTCTTTCGTATCTGAATTTCCACGTTTATTAAGCAATGT		121
Sbjct 164786		GCGTTGAATCAAAGCAATACGCTCTTTCGTATCTGAATTTCCACGTTTATTAAGCAATGT		164845
Query 122		CAAAGGATAGGTATCGCTATTCCACGATAAACGATTGCGAGCATCAGGATATTAATATT		181
Sbjct 164846		CAAAGGATAGGTATCGCTATTCCACGATAAACGATTGCGAGCATCAGGATATTAATATT		164905
Query 182		TCGTTTTCCCATTTCCAATTGGTTCATCTAAAGTCAGTTGCACTTGGTCGAATGAAAA		241
Sbjct 164906		TCGTTTTCCCATTTCCAATTGGTTCATCTAAAGTCAGTTGCACTTGGTCGAATGAAAA		164965
Query 242		CATATTGAAAATCAACTGAGAAATTTGACGATAATCAAAATACTGACCTGCAAAGAAGCG		301
Sbjct 164966		CATATTGAAAATCAACTGAGAAATTTGACGATAATCAAAATACTGACCTGCAAAGAAGCG		165025
Query 302		CTGCATACGTCGATAAAAATGATTGTGGTAAGCACTTGATGGGCAAGGCTTTAGATGCAGA		361
Sbjct 165026		CTGCATACGTCGATAAAAATGATTGTGGTAAGCACTTGATGGGCAAGGCTTTAGATGCAGA		165085
Query 362		AGAAAGATTACATGTTTGCTTTAAATAATCACAAGCATGATGAGCGCAAAGCACTTTAA		421
Sbjct 165086		AGAAAGATTACATGTTTGCTTTAAATAATCACAAGCATGATGAGCGCAAAGCACTTTAA		165145
Query 422		ATGTGACTTGTTCATTTTAGAGATTTGTTTAAGATAAGATATAAATCATTGAGATGTGT		481
Sbjct 165146		ATGTGACTTGTTCATTTTAGAGATTTGTTTAAGATAAGATATAAATCATTGAGATGTGT		165205
Query 482		C-T-GTATT 488		
Sbjct 165206		CATAGTATT 165214		

Figure 4. Sequence alignment of ISAba1(Forward) of *A.baumannii*(X10 isolate) with *Acinetobacter baumannii* strain VB473 chromosome, from India.

20. Brooks G F, Carroll KC, Butel JS, Morse S A, Miettinen, T.A. Medical Microbiology. Jawetz, Melnick and adelberg's. McGraw-Hill Companies, Inc. USA 2013; 26th ed.

21. Ling T K, Liu Z K, Cheng A F. Evaluation of the vitek 2 system for rapid direct identification and susceptibility testing of gram-negative bacilli from positive blood cultures. Journal of clinical microbiology. 2003; 41(10): 4705-4707.

22. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.

23. Asif M, Alvi IA, Rehman SU. Insight into *Acinetobacter baumannii* : pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities. Infect Drug Resist. 2018;11:1249-1260

24. Kempf M, Rolain JM. Emergence of resistance to carbapenems in *Acinetobacter baumannii* in Europe: clinical impact and therapeutic options. Int J Antimicrob Agents. 2012; 39: 105-114. PMID:22113193.

25. Higgins PG, Dammhayn C, Hackel M, Seifert H. Global spread of carbapenem-resistant *Acinetobacter baumannii*. J Antimicrob Chemother. 2010;65(2):233-8.

26. Fatima S. Assessment correlation between biofilm formation and type II toxin-antitoxin system in imipenem-resistant *Acinetobacter baumannii*. Submitted to the Council of College of Science / Mustansiriyah University In Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy (Ph.D.) in Biology/ Microbiology. 2018

27. Owrang M, Fallah F, Irani S, Rahbar M, Eslami G. Identification and Isolation of Insertion Sequences, in Carbapenem Resistant Clinical Isolates of *Acinetobacter baumannii* from Tehran Hospitals, Jundishapur J Microbiol. 2018 ; 11(6):e58251. doi: 10.5812/jjm.58251
28. McConnell MJ, Domínguez-Herrera J, Smani Y, López-Rojas R, Docobo-Pérez F, Pachón J. Vaccination with outer membrane complexes elicits rapid protective immunity to multidrug-resistant *Acinetobacter baumannii*. Infect Immun. 2011;79:518–26.
29. Turton JF, Ward M E, Woodford N, Kaufmann M E, Pike R, Livermore D M, Pitt T L. The role of IS*Aba*1 in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. FEMS Microbiol. Lett. 2006b; 258(1):72–77.
30. Evans B A, Hamouda A, Towner K J, Amyes S G. OXA-51-like beta-lactamases and their association with particular epidemic lineages of *Acinetobacter baumannii*. Clin. Microbiol. Infect. 2008;14(3): 268–275.
31. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. Int J Antimicrob Agents. 2006;27(4):351–3.
32. Alexandre P Zavascki, Cecília G Carvalhaes, Renata C Picão & Ana C Gales. Multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: resistance mechanisms and implications for therapy, Expert Review of Anti-infective Therapy.2010; 8:1, 71–93.
33. Sohrabi B, Vanani IR, Tahmasebipour K, Fazli S. An exploratory analysis of hotel selection factors: A comprehensive survey of Tehran hotels. Int J Hosp Manag. 2012;31(1):96–106.
34. Ghaima K K, Saadedin S M K, Jassim K A. Isolation, molecular identification and antimicrobial susceptibility of *Acinetobacter baumannii* isolated from Baghdad. Int. J. Sci. Res. Publ. 2016; 6(5): 351–356.
35. Hussein N H, Al-Mathkhury H J F, Sabbah M A. Imipenem-Resistant *Acinetobacter baumannii* isolated from patients and hospitals environment in Baghdad. Iraqi J. Sci. 2013; 54(4): 803–812
36. Abouelfetouh A, Torky AS, Aboulmagd E. Phenotypic and genotypic characterization of carbapenem-resistant *Acinetobacter baumannii* isolates from Egypt. Antimicrob Resist Infect Control. 2019;8(1):1–9
37. Bahador A, Raoo An, R Farshadzadeh Z, Beitollahi L, Khaledi A, Rahimi S, Mokhtaran M, Mehrabi Tavana A, Esmaeili D. The Prevalence of IS *Aba* 1 and IS *Aba* 4 in *Acinetobacter baumannii* Species of Different International Clone Lineages Among Patients With Burning in Tehran, Iran. Jundishapur journal of microbiology.2015; 8(7), e17167.
38. Alsultan AA, Aboulmagd E, Evans BA, Amyes SG. Clonal diversity of *Acinetobacter baumannii* from diabetic patients in Saudi Arabian hospitals. J Med Microbiol. 2014;63(11):1460–6.
39. Villalón P., Valdezate S., Medina-Pascual M.J., Carrasco G., Vindel A., Saez-Nieto J. A. 2013; Epidemiology of the *Acinetobacter*-derived cephalosporinase, carbapenem-hydrolysing oxacillinase and metallo-β-lactamase genes, and of common insertion sequences, in epidemic clones of *Acinetobacter baumannii* from Spain. J Antimicrob Chemother 68:550–553
40. Lu P L, Doumith M, Livermore D M, Chen T P, Woodford N. Diversity of carbapenem resistance mechanisms in *Acinetobacter baumannii* from a Taiwan hospital: spread of plasmid-borne OXA-72 carbapenemase. J Antimicrob Chemother. 2009; 63:641–647
41. Karunasagar A, Maiti B, Shekar M, Shenoy M S, Karunasagar I. Prevalence of OXA type carbapenemase genes and genetic heterogeneity in clinical isolates of *Acinetobacter* spp. from Mangalore, India. Microbiol Immunol. 2011;55(4):239–46.
42. Lee K, Yong D, Jeong SH, Chong Y. Multidrug-resistant *Acinetobacter* spp.: increasingly problematic nosocomial pathogens. Yonsei Med J. 2011;52(6):879–91.

Received: 7 August 2021

Accepted: 21 September 2021