[Downloaded free from http://www.mmjonweb.org on Monday, July 4, 2022, IP: 37.237.24.42]

Original Article

Identification and Sequencing of *ISAba2* of *Acinetobacter baumannii* Isolated from Baghdad Hospitals

Salah Sabah Muhsin[1], Wasan Abdul-Elah Bakir[2], Majeed Arsheed Sabbah[3]

[1]College of Science, 2012, Baghdad University, [2]Department of Microbiology, College of Medicine, Mustansiriyah University, Baghdad, Iraq, [3]Department of Gene Bank, Forensic DNA for Research and Training Centre, Al-Nahrain University, Baghdad, Iraq

Abstract

Background: The ability of multidrug-resistant *Acinetobacter baumannii* to survive in any situation including the acquisition of many different kinds of virulence factors and antibiotic resistance genes is the primary source of worry in hospital settings. Despite their low hydrolysis capability, oxacillinase (OXA) types are often associated with genetic factors such as insertion sequences (ISs) in order to enhance carbapenemase production and mobilization. As a result, assessing the frequency of IS genes in *A. baumannii* is extremely essential in many hospitals and medical institutions. **Aims:** The goal of this work is to find the IS *ISAba2*, which may have a role in antibiotic resistance in extensively drug-resistant (XDR) *A. baumannii*. **Methods:** The polymerase chain reaction (PCR) was used to confirm the presence of *A. baumannii* by identifying the bla_{OXA-51} gene. According to the current Clinical and Laboratory Standards Institute recommendations (2020), antimicrobials are determined using the Kirby–Bauer disc diffusion technique on Mueller-Hinton agar. The molecular research of *ISAba2* includes PCR and Sanger sequencing of the PCR results. **Results:** Among 38 *A. baumannii* isolates, 23 (61%) and 25 (66%) were resistant to meropenem and imipenem, respectively. The bla_{OXA-51} gene was detected in all 21 XDR strains tested; furthermore, *ISAba2* was found in all 21 XDR-analyzed *A. baumannii* isolates. **Conclusions:** *ISAba2* has a high predominance between extreme drug-resistant *A. baumannii*. The identification of these parameters can assist in the control of infection and decrease of the microorganism's prevalence rate.

Keywords: Insertion sequences, mobile genetic elements, multidrug resistant (extensively drug resistant)

Introduction

Acinetobacter baumannii is a common opportunistic pathogen among immunocompromised persons, especially those who have remained more than 90 days in the hospital.[1] Commonly associated with aquatic environments.[2] It has been recognized as a "red alert" human pathogen, causing concern among medical professionals due to its wide range of antibiotic resistance.[3]

The development of multidrug-resistant (MDR) microorganisms has become a major concern in the context of both nosocomial and community-acquired illnesses.[4] According to the World Health Organization, antibiotic resistance is one of the three most serious challenges confronting human health today.[5] The therapeutic options are restricted, which frequently leads to ineffective treatment and unfavorable effects for patients.[6]

Enzymatic (production of β-lactamases and enzymatic modification of aminoglycosides) and nonenzymatic processes (changes in membrane permeability, efflux pump activation, and target site modification) are the major mechanisms of antibiotic resistance.[7]

In all species, insertion sequences (ISs) constitute basic mobile genetic elements (MGEs). They may travel horizontally across genomes as part of other MGE vectors such as phages and plasmids, or vertically inside a genome as part of other MGE vectors such as phages and plasmids (between different DNA molecules or within a specific DNA molecule).[8,9] So far, approximately 4500 ISs from 29 families have been found.[9] Resistance is aided by the insertional inactivation of proteins such as transcriptional regulators and outer membrane proteins. In

Access this article online

Quick Response Code:

Website: http://www.mmjonweb.org

DOI: 10.4103/mj.mj_17_21

Address for correspondence: Mr. Salah Sabah Muhsin, B.Sc. of Biology, College of Science, Baghdad University 2012, Baghdad, Iraq.
E-mail: guneydenguneyden@gmail.com

Submitted: 21-Aug-2021 **Revised:** 17-Oct-2021 **Accepted:** 25-Oct-2021 **Published:** 30-Jun-2022

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Muhsin SS, Bakir WA, Sabbah MA. Identification and sequencing of *ISAba2* of *Acinetobacter baumannii* isolated from baghdad hospitals. Mustansiriya Med J 2022;21:29-36.

[Downloaded free from http://www.mmjonweb.org on Monday, July 4, 2022, IP: 37.237.24.42]

Acinetobacter spp., more than 30 IS types have been discovered, confirming that ISs are significant in the development of this species and have contributed to MDR phenotypes.[10]

ISs are short DNA sequences that act as simple transposable elements. ISs have two different features, and they are short (up to 2500 bp). Insertion Sequences have two different features, are short (up to 2500 bp) and only code for proteins that are involved in the transposition process. The transposase, which catalyzes the enzymatic process that allows the IS to transfer, and one regulatory protein, which either enhances or suppresses the transposition activity, are generally the proteins involved.[11]

IS *ISAba2* is associated with resistance genes $bla_{\text{OXA-58}}$ and *blaAmpC*.[12] Therefore, determining the prevalence of *ISAba2* in *A. baumannii* in different hospitals and medical centers is very important.

Methods

Specimen collection

A total of 540 different clinical specimens were collected during the period between October 2020 and February 2021 including sputum, blood, fluids (cerebrospinal, pleural, and peritoneal), urine, and swabs from five hospitals in Baghdad including the Burn Teaching Hospital, the Martyr Ghazi Al-Hariri Hospital, Baghdad Teaching Hospital, Welfare Teaching Hospital in the Medical City, and Al-Yarmouk Teaching Hospital.

Isolation of bacteria

In the laboratory (Teaching Laboratories/Medical City, Baghdad), the obtained specimens were cultured directly on blood agar and MacConkey agar under aseptic circumstances and incubated for 24 h at 37°C. The nonhemolytic opaque creamy colonies on blood agar and nonlactose-fermenting colonies on MacConkey agar were subcultured on another MacConkey agar plate and incubated for another 24 h at 37°C to obtain pure well-isolated colonies.[13]

Bacterial identification

Microscopical examination

One isolated colony was transferred to a microscopic slide, fixed, and then stained with Gram stain. Gram reaction, cell shape, and arrangement were recorded. The results were compared with Brooks *et al*.[14]

Biochemical tests

Biochemical assays such as growth at 42°C, culture on selective media, negative oxidase test, absence of lactose fermentation, and others were used to phenotypically identify the isolates as *A. baumannii*.

Identification of *Acinetobacter baumannii* using VITEK® 2 system

Bacterial isolates were identified at species level by using VITEK® 2 system with Identification-Gram Negative Bacteria (ID-GN) cards according to the manufacturer's instructions. A single colony of the bacterial isolates (previously incubated at 37°C for overnight on MacConky agar plates) were taken then suspended in 0.45% sodium chloride. The turbidity of bacterial suspension was measured to match the McFarland (0.5) standard, then the (Gram Negative VITEK® 2 ID card) with the bacterial suspension tubes were loaded manually into the VITEK® 2 system, the software also prepared according to (BioMerieux, France) the manufacturer's instructions.[15]

Identification of *Acinetobacter baumannii* by polymerase chain reaction

Polymerase chain reaction (PCR) was done to amplify $bla_{\text{OXA-51}}$-like genes, which was used for the identification of *A. baumannii* isolates by using a primer specific for $bla_{\text{OXA-51}}$-like genes.

Antimicrobial susceptibility test

A panel of antimicrobial susceptibility testing of the isolates to 18 agents was determined by Kirby–Bauer disc diffusion method on Mueller-Hinton agar (Oxoid/England) according to the current Clinical and Laboratory Standards Institute guidelines (2020).[16] The antibiotic discs (Mast Group/UK) used throughout the study for *A. baumannii* isolates are listed in Table 1.

Extraction of genomic DNA from bacterial isolates

DNA was extracted from all bacterial isolates under study using a commercial purification system ZR Fungal/Bacterial DNA MiniPrep Kit designed for the isolation of DNA from Gram-negative bacteria according to the manufacturer's instructions. A total of 4 µL of DNA extract was used for each reaction.

Molecular recognition of $bla_{\text{OXA-51}}$-like gene and insertion sequence elements

For the detection of extensively drug-resistant (XDR) *A. baumannii* isolates, PCR was used for identification of the $bla_{\text{OXA-51}}$-like gene and *ISAba2* elements. Primer sequences for each gene reported above are listed in Table 1. These primers (Macrogen, South Korea) were received in lyophilized state, dissolved in sterile deionized distilled water to a final

Table 1: Sequences of primers used throughout the study

Primer	Sequence of primers (5'---3')	Product size (bp)	Accession number
OXA51 F	CTTTTTGGCTAAATGGAAGCG	434	CP081137.1
OXA51 R	CGGGTGTCTTAGTTATCCAAC		
ISaba2 F	TACCCCTGAAATCCGAGATAG	372	CP029569.1
ISaba2 R	GCGATCGGTAAAATCCTACAA		

OXA: Oxacillinase

[Downloaded free from http://www.mmjonweb.org on Monday, July 4, 2022, IP: 37.237.24.42]

concentration of 100 picomole/µl, and kept in a deep freezing until use, as advised by the vendor.

The PCR amplification procedure for the genetic level to detect genes under study included the following steps: final volume for PCR mixture was 25 µl (12.5 µl of Green Master Mix 2x, 4 µl extracted template DNA, 1.5 µl from each forward and reverse primer, 5.5 µl nuclease-free water) were added in 0.2 ml PCR Eppendorf tubes, mixed for a short time via vortex then have 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 bla_{OXA-51}-like gene and *ISAba2*, the DNA thermal cycler device Veriti™ 96-Well (Applied Biosystems) was programmed in the following amplification conditions: following a 5-min activation at 94°C, 40 cycles of 45 s at 94°C (denaturation), 56°C (annealing), and 45 s at 72°C (extension) were conducted. The last cycle was followed by a 7-min period at 72°C [Table 2]. Amplified PCR products were examined by 1.5% agarose gel at an electrical current of 7 volt\cm2 in 1X TBE buffer with added RedSafe dye (iNtRON Biotechnology/Korea) has been exposed till the tincture reached the other side of the gel. The SiZer™-1000 Plus DNA Marker and SiZer™-100 DNA Marker (iNtRON Biotechnology/Korea) were used as a marker during PCR products electrophoresis. Thereafter, the agarose gel was removed from the tank and visualized by ultraviolet transilluminator documentation system (Cleaver Scientific/UK) with 336 nm and 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). One specimen of *ISAba2* PCR products with forward primer (17 pmol/µl) for the gene was 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 have 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.

Statistical analysis

The IBM SPSS Statistics 24 (IBM Corp., Armonk, NY) and Microsoft® Excel® 2019 MSO software were used to analyze the data. The Chi-square test was used to establish a significant comparison between percentages (0.05 and 0.01 probability in this research).

Table 2: Programs were used in the polymerase chain reaction for oxacillinase-51 and *ISaba2* genes

Steps	OXA-51	*ISAba2*	Repeats
Initial denaturation	94°C/5 min	94°C/5 min	1 cycle
Denaturation	94°C/45 s	94°C/45 s	40 cycles
Annealing	56°C	56°C	
Extension	72°C/45 s	72°C/45 s	
Final extension	72°C/7 min	72°C/7 min	-

OXA: Oxacillinase

Results

A total of 540 different clinical specimens were collected between October 2020 and November 2021 from some hospitals in Baghdad city including Baghdad Medical City and Al-Yarmouk Hospital. Preliminary identification of isolates is done through cultural identification, microscopic examination, and biochemical tests. All nonlactose fermenter isolates were also identified at species level with VITEK® 2 system by using ID-GNB cards, and the results showed that isolates under study belong to *A. baumannii.* Thirty-eight bacterial isolates were collected, all of which were identified as *A. baumannii,* as revealed by Figure 1.

Finally, all the 21 XDR *A. baumannii* isolates were also identified depending on a bla_{OXA-51}-like genes (carbapenem resistance genes), and all isolates gave a positive result.

Thirty-eight bacterial isolates were obtained from various clinical specimens including 12 isolates (32%) from sputum, 11 (29%) swabs, 8 (21%) urine, 5 (13%) blood, and 2 (5%) fluid, as shown in Figure 2.

The gender distribution of the infected patients with *A. baumannii* is summarized in Figure 3. The incidence was higher among males 58% than that of females 42%. Table 3 shows the distribution of *A. baumannii* Isolates according to the source and gender.

An assessment was then made of the relative numbers of *A. baumannii* isolates according to the type of specimen with respect to gender. Males differ significantly in terms of the number of *A. baumannii* in either specimen (P = 0.0001). However, in the female group, the differences were also highly significant (P = 0.0001), as illustrated in Table 3.

By observing the results in Table 4, *A. baumannii* show a high level of resistance of clinical isolates to most of the antibiotics under test. In this study, antibiotic resistance profile of *A. baumannii* for 18 antibiotics as follows: trimethoprim/sulfamethoxazole 32 (84%), ciprofloxacin 27 (71%), piperacillin/tazobactam 26 (68%), ticarcillin/clavulanate 26 (68%), ceftazidime 26 (68%), ceftriaxone 26 (68%), cefotaxime 25 (66%), imipenem 25 (66%), doxycycline 26 (68%), levofloxacin 25 (66%), cefepime 24 (63%),

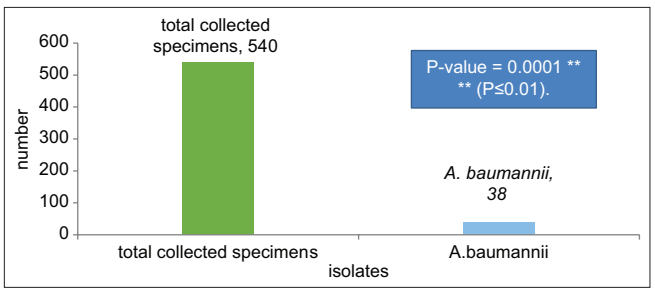

Figure 1: Frequency of clinical specimens that give positive result of *Acinetobacter baumannii* from all isolated specimens

[Downloaded free from http://www.mmjonweb.org on Monday, July 4, 2022, IP: 37.237.24.42]

tetracycline 24 (63%), meropenem 23 (61%), gentamicin 22 (58%), amikacin 17 (45%), tobramycin 15 (39%), ampicillin/sulbactam 12 (32%), and colistin 0 (0%).

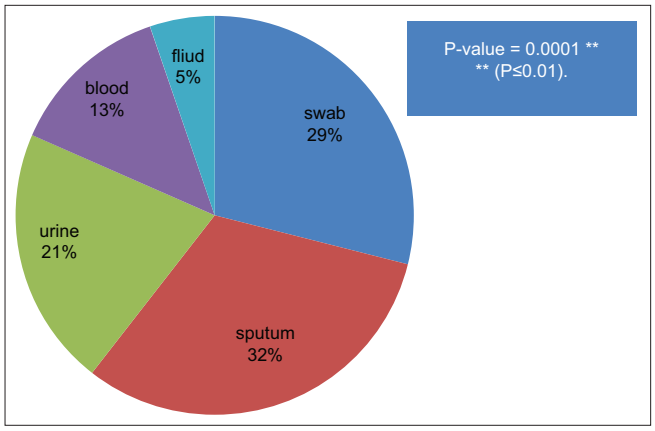

Figure 2: Distribution of *Acinetobacter baumannii* isolates according to type of positive specimen from total 38 positive isolates of *Acinetobacter baumannii*

Table 3: Distribution of *Acinetobacter baumannii* isolates according to the source and gender

Specimen	Total number	Male, *n* (%)	Female, *n* (%)
Sputum	12	6 (50)	6 (50)
Swab	11	7 (64)	4 (36)
Urine	8	4 (50)	4 (50)
Blood	5	3 (60)	2 (40)
Fluid	2	2 (100)	0
Total	38	22	16
P	-	0.0001**	0.0001**

**$P \leq 001$

Resistance of *A. baumannii* clinical isolates to the tested antibiotics showed that from 38 isolates, 32 (84%) were resistant to more than at least one antibiotic in three or more than three antibiotic groups. Consequently, it was crucial to classify all *A. baumannii* isolates into two major categories including non-MDR (N-MDR) and MDR, MDR classified into three categories including MDR, XDR, and pandrug resistant (PDR). Classification was done according to the criteria mentioned by Magiorakos *et al*.[17] Thus, all 38 *A. baumannii* isolates were classified as MDR in the first place; however, the majority of them were converted to be XDR or even PDR depending on their resistance profile and as follows: there were only six isolates (16%) of *A. baumannii* isolates that were considered as N-MDR since they were susceptible to all antimicrobial categories but resistant to some, not all agents listed in the extended-spectrum cephalosporin category. There were 32 isolates (84%) of *A. baumannii* isolates that were classified as MDR since they were resistant to ≥3 out of all antimicrobial categories that match the criteria used. It should be noted that even the isolate could resist to at least one agent in three or more antimicrobial categories; it would be considered resistant isolate to that category. There were 21 isolates (66%) of *A. baumannii* isolates that were considered as XDR since they were susceptible to only 2 out of all antimicrobial categories that match the criteria used. Thus, XDR was defined as nonsusceptibility to at least one agent in all but remained susceptible to only one or two antimicrobial categories. There were no isolates that show PDR pattern.

Genomic DNA was successfully extracted from all bacterial isolates by using genomic DNA purification kit, and the DNA

Table 4: Frequency and percentage of resistance *Acinetobacter baumannii* against antimicrobial agents

Antimicrobial agent	Resistant, *n* (%)	Intermediate, *n* (%)	Sensitive, *n* (%)	*P*
Trimethoprim/sulfamethoxazole	32 (84)	2 (5)	4 (11)	0.0001**
Ciprofloxacin	27 (71)	1 (3)	10 (26)	0.0001**
Piperacillin/tazobactam	26 (68.4)	7 (18.4)	5 (13.2)	0.0001**
Ticarcillin/clavulanate	26 (68)	3 (8)	9 (24)	0.0001**
Ceftazidime	26 (68)	4 (11)	8 (21)	0.0001**
Ceftriaxone	26 (68)	4 (11)	8 (21)	0.0001**
Cefotaxime	25 (66)	7 (18)	6 (16)	0.0001**
Imipenem	25 (66)	5 (13)	8 (21)	0.0001**
Doxycycline	26 (68)	2 (5)	10 (26)	0.0001**
Levofloxacin	25 (66)	3 (8)	10 (26)	0.0001**
Cefepime	24 (63)	4 (11)	10 (26)	0.0001**
Tetracycline	24 (63)	7 (18)	7 (18)	0.0001**
Meropenem	23 (61)	1 (3)	14 (36)	0.0001**
Gentamicin	22 (58)	0	16 (42)	0.0001**
Amikacin	17 (45)	7 (18)	14 (37)	0.0074**
Tobramycin	15 (39)	4 (11)	19 (50)	0.0069**
Ampicillin/sulbactam	12 (32)	10 (26)	16 (42)	0.0277**
Colistin	0	0	38 (100)	0.0001**
P	0.0001**	0.0072**	0.0001**	-

**$P \leq 001$

[Downloaded free from http://www.mmjonweb.org on Monday, July 4, 2022, IP: 37.237.24.42]

bands were confirmed and analyzed by gel electrophoresis. Then, the purity of extracted DNA was determined between 1.7 and 2.

The 21 XDR *A. baumannii* isolates were identified to species level depending on carbapenem-hydrolyzing genes (bla_{OXA-51}-like) (oxacillinases [OXAs]) belonging to class D β-lactamase. Each DNA extracted sample was subjected to a monoplex PCR reaction with a primer of this gene. All isolates gave a positive result, and the PCR products have been confirmed by comparing its base-pair size (434 bp) with SiZer-100 DNA Marker and SiZer™-1,000 plus DNA Marker, as shown in Figure 4. One of the present study objectives is to detect the existence and prevalence of *ISAba2* genes. Each DNA extracted sample was subjected to monoplex PCR reaction with specific primer sets of *ISAba2* genes. The PCR products have been confirmed by comparing its base-pair size (372 bp) with SiZer-100 DNA ladder, the results presented in Table 5, showed that the presence of *ISAba2* gene in all (100%) XDR *A. baumannii* isolates, as shown in Figure 5.

Monoplex PCR products for genes under study were detected by agarose gel electrophoresis and then the sequencing was carried out by (Macrogen Research, Seoul, Korea). Aligning of the obtained sequences with those of reference strains in GenBank confirmed the correct identification of these genes by PCR at (99%) for sequenced strand. The sequence was analyzed by BLAST software in NCBI. Figure 6 shows the alignment result with Indian isolate (Accession number (CP050415.1). The sequence was found to share 99% nucleotide homology with the reference isolates. Tables 6 and 7 show the nucleotide changes and features of *ISAba2* (Forward) from *A. baumannii* (X7 isolate) with *A. baumannii* strain PM193665 chromosome from India.

Table 5: Number and percentage of *ISAba2* genes for all extensively drug-resistant *Acinetobacter baumannii* clinical isolates

Total number of isolates	(+V) *ISAaba2*, *n* (%)	(−V) *ISAba2*, *n* (%)
21 XDR *A. baumannii*	21 (100)	0
P	0.0001**	

**$P \leq 001$. XDR: Extensively drug-resistant, +V: Positive result, −V: Negative result, *A. baumannii*: *Acinetobacter baumannii*

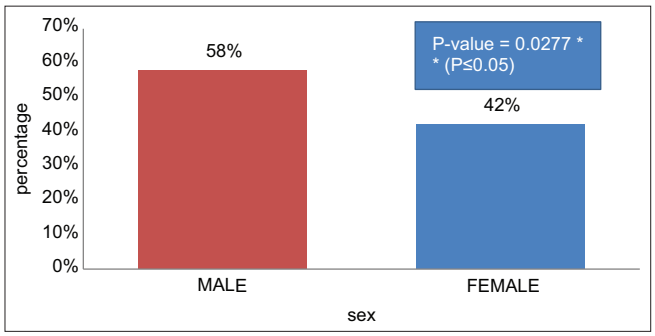

Figure 3: Distribution of *Acinetobacter baumannii* isolates according to gender

Discussion

A. baumannii has developed as a well-established nosocomial pathogen with a high degree of antibiotic resistance. Due to rising resistance rates, carbapenems, the medication of choice for treating *A. baumannii* infections, are becoming more ineffective.[18] Carbapenem resistance is often linked to the development of OXA enzymes. Metallo-β-lactamases, on the other hand, may make *A. baumannii* resistant to carbapenems.[19]

This study out of 540 different clinical specimens shows that the highest isolation was from sputum and swab specimens more than other sources, followed by urine specimens, and the low percentage was in the blood and fluid specimens. In a local study, 170 bacterial isolates were obtained from various clinical specimens including 53 isolates (31.18%) from sputum, 45 (26.47%) wound, 43 (25.29%) blood, 12 (7.06%) burn, 10 (5.88%) urine, 5 (2.94%) fluid, and 2 (1.18%) ear swab.[20]

Another study in Iraq revealed that *A. baumannii* was isolated in high percentage 44 (38.26%) from sputum specimens; while, blood specimens constituted 30 (26.09%), wound specimens achieved 27 (23.48%), urine specimens formed 8 (6.95%), and the low percentage was in burn specimens which accomplished 6 (5.22%).[21] The variations in isolation percentage of pathogenic bacteria in studies may be attributed to differences in many factors such as sanitary practices for hospital staff, environmental conditions, isolation and identification techniques, social and cultural level of patients, differences in the prevalence of infection from one country to another, and difference in patients, which may inhibit or stimulate the growth and distribution of bacteria in hospitals.[22]

The incidence of infection was higher among males than that of females. These results agree with another study.[23]

According to this study, significantly high resistance rates to Trimethoprim/sulphamethoxazole and Ciprofloxacin, and

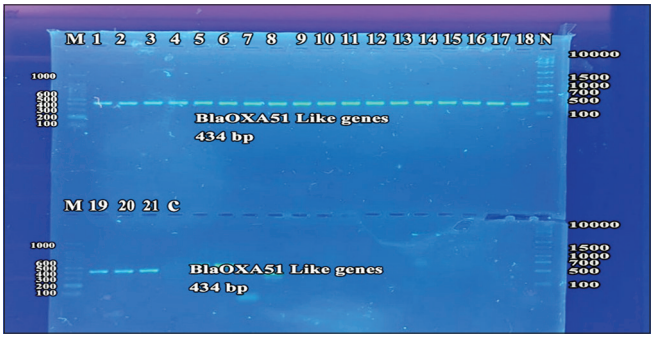

Figure 4: Detection of bla_{OXA-51}-like gene by monoplex polymerase chain reaction for *Acinetobacter baumannii* isolates. Lanes 1-21, XDR *Acinetobacter baumannii*; Lane C, Negative control. Lane M, 100 bp DNA marker. Lane N, 1000 bp plus DNA marker. Detection was done on agarose gel (1.5%) at 5 V/cm for 1.5 h, stained with RedSafe dye, and visualized on a ultraviolet transilluminator documentation system

[Downloaded free from http://www.mmjonweb.org on Monday, July 4, 2022, IP: 37.237.24.42]

Table 6: Nucleotide changes of *ISAba2* (forward) from *Acinetobacter baumannii* (X7 isolate) with *Acinetobacter baumannii* strain PM193665 chromosome from India

Gene: *ISAba2* (forward) from *A. baumannii* (X7 isolate)					
Type of substitution	**Location**	**Nucleotide**	**Sequence ID with compare**	**Source**	**Identities (%)**
Transition	288	G/A	CP050415.1	*A. baumannii*	99

A. baumannii: *Acinetobacter baumannii*

Table 7: Features of *ISAba2* (forward) from *Acinetobacter baumannii* (X7 isolate) with *Acinetobacter baumannii* strain PM193665 chromosome, from India

Feature	Studied isolate	Reference isolate
Molecule type	Genomic DNA	Genomic DNA
Isolation source	Swab	Pus
Host	*Homo sapiens*	*Homo sapiens*
Country	Iraq, Baghdad	India

moderate resistance to Piperacillin/tazobactam, Ticarcillin/clavulanate, Ceftazidime, Ceftriaxone, Doxycycline, Cefotaxime, Levofloxacin, Cefepime, Tetracycline, and Gentamicin. Low resistance rates were recorded to Amikacin, Tobramycin, and Ampicillin/sulbactam and no resistance to Colistin (0%). The important findings of the current study indicated that the 38 A. baumannii isolates were moderately resistant to meropenem and imipenem. On the other hand, the significantly high degree of sensitivity rates to tobramycin 50% and colistin 100% w detected.

The findings of this study corroborated the findings of previous studies on *A. baumannii* resistance to the majority of antibiotics, indicating that these bacteria have a wide range of resistance mechanisms. This would imply that there are significant risks among hospitalized patients, particularly where this antibiotic class was previously thought to be the usual therapy for *A. baumannii* infections.[24]

Results of resistance of *A. baumannii* clinical isolates to the tested antibiotics showed a high percentage of MDR isolates, while few isolates were N-MDR. Furthermore, a high number of isolates were XDR and no isolate was PDR. These results agreed with another study found that from 102 isolates, 95 (93.14%) isolates were resistant to more than two classes of antibiotics (MDR).[25] Thus, bacterial isolates that are characterized as XDR will also be characterized as MDR. Similarly, bacterial isolates would have to be XDR for them to be further defined as PDR. The recognition of the $bla_{OXA\text{-}51}$-like gene can be utilized to identify *A. baumannii* simply and reliably.[26,27]

Furthermore, because this gene was controlled by ISs such as *ISAba1*, the presence of intrinsic chromosomally placed genes of the $bla_{OXA\text{-}51}$-like gene did not correlate with the amount of carbapenem resistance of *A. baumannii* isolates.[28] The results of the current study revealed that $bla_{OXA\text{-}51}$-like genes are found in all 21 studied XDR *A. baumannii* isolates. The findings of this study corroborated those of previous

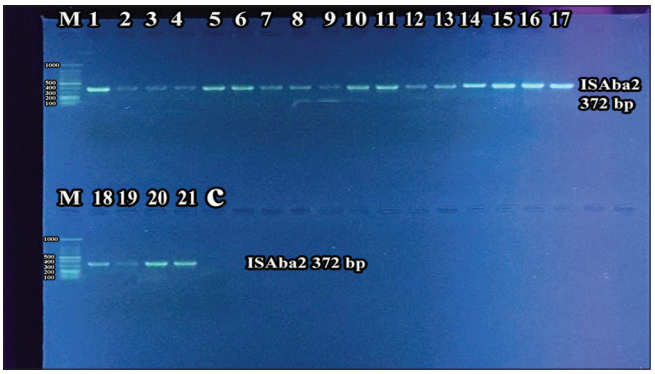

Figure 5: Detection of *ISAba2* gene by monoplex polymerase chain reaction of isolates. Lanes 1-21, XDR *Acinetobacter baumannii* isolates. Lane C, Negative control. Lane M, 100 bp DNA ladder. Detection was done on agarose gel (1.5%) at 5 V/cm for 1.5 h, stained with RedSafe dye, and visualized on a ultraviolet transilluminator documentation system

local investigations and revealed that all 127 *A. baumannii* isolates were positive for $bla_{OXA\text{-}51}$-like gene.[20] Another local study discovered that the $bla_{OXA\text{-}51}$-like gene is present in all 96 isolates diagnosed as *A. baumannii* based on PCR data.[29] Another study in Egypt found that genes encoding for $bla_{OXA\text{-}51}$ belonging to class D carbapenemases were found in 100% of the isolates.[30]

The results of this study revealed that all 21 (100%) XDR isolates carry the *ISAba2* gene. A study in Iran found that the prevalence rate of ISAba2 in five hospitals was 92.38%, and in three of them (Taleghani, Milad, and Mofid), the prevalence rate was 100%,[23] but in Egypt, found that the prevalence of ISAba2 was 2.7%.[30]

Conclusions

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 inpatients 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 certain mobile components that can be transported between species to change the antimicrobial pattern and enhance antimicrobial resistance. *ISAba2* has a high prevalence among extreme drug-resistant *A. baumannii* isolated from several Baghdad hospitals. The identification of these

[Downloaded free from http://www.mmjonweb.org on Monday, July 4, 2022, IP: 37.237.24.42]

Score	Expect	Identities	Gaps	Strand
532 bits (288)	5e-147	290/291 (99%)	0/291 (0%)	Plus/Minus

```
Query  2        GCTGCGATCACCGCTATTGCTCCCAAGATAGGTTGTACTCCTGAAACACTACGTGTTTGG  61
                |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3611308  GCTGCGATCACCGCTATTGCTCCCAAGATAGGTTGTACTCCTGAAACACTACGTGTTTGG
3611249

Query  62       TATCAAAAATATTTAGATAAACTAAATCCAGTTAAAGTACAGCAGCTTTCAGATCAAGAA  121
                |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3611248  TATCAAAAATATTTAGATAAACTAAATCCAGTTAAAGTACAGCAGCTTTCAGATCAAGAA
3611189

Query  122      CGTATTAAACAACTCGAACGTGAAAATAAAGAACTGCAACGCGCCAATGAGATTCTACGT  181
                |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3611188  CGTATTAAACAACTCGAACGTGAAAATAAAGAACTGCAACGCGCCAATGAGATTCTACGT
3611129

Query  182      AAAGCAGCCGCTTTTTTCGCCCAGGCGGAGCTCGACCGCCCACACAAATAATGGTGGATT  241
                |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3611128  AAAGCAGCCGCTTTTTTCGCCCAGGCGGAGCTCGACCGCCCACACAAATAATGGTGGATT
3611069

Query  242      TTATCCATAATAATAAAGAGCTGTACGGAGTCGAGGCGATTTGTAGGATTT  292
                |||||||||||||||||||||||||||||||||||||||||||| ||||
Sbjct  3611068  TTATCCATAATAATAAAGAGCTGTACGGAGTCGAGGCGATTTGTAGAATTT  3611018
```

Figure 6: Sequence alignment of *ISAba2* (forward) of *Acinetobacter baumannii* (X7 isolate) with *Acinetobacter baumannii* strain PM193665 chromosome from India. Sequence ID: CP050415.1, length: 3948262. Range 1: 3611018 to 3611308

parameters can aid in the control of infection and the reduction of the microorganism's prevalence rate.

Recommendations

Whole genome sequencing of Acinetobacter baumannii to determine the number and distribution of *ISAba2* in the whole genome. Study the effects of *ISAba2* insertion on the antibiotic resistance in A. baumannii. Determine the relationship between *ISAba2* with β-lactamase encoding genes. Study the amino acid variation of *ISAba2* in local A. baumannii isolates in comparison to reference isolates, as well as the effect on the resultant protein.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. 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;28:15-25.
2. Turton JF, Ward ME, Woodford N, Kaufmann ME, Pike R, Livermore DM, *et al.* The role of ISAba1 in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. FEMS Microbiol Lett 2006;258:72-7.
3. Cerqueira GM, Peleg AY. Insights into *Acinetobacter baumannii* pathogenicity. IUBMB Life 2011;63:1055-60.
4. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: Emergence of a successful pathogen. Clin Microbiol Rev 2008;21:538-82.
5. Bassetti M, Ginocchio F, Mikulska M. New treatment options against gram-negative organisms. Crit Care 2011;15:215.
6. Rice LB. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: No ESKAPE. J Infect Dis 2008;197:1079-81.
7. Vrancianu CO, Gheorghe I, Czobor IB, Chifiriuc MC. Antibiotic resistance profiles, molecular mechanisms and innovative treatment strategies of *Acinetobacter baumannii*. Microorganisms 2020;8:E935.
8. Siguier P, Filée J, Chandler M. Insertion sequences in prokaryotic genomes. Curr Opin Microbiol 2006;9:526-31.
9. Siguier P, Gourbeyre E, Chandler M. Bacterial insertion sequences: Their genomic impact and diversity. FEMS Microbiol Rev 2014;38:865-91.
10. Montaña SD, Almuzara M, Pennini M, Sucari A, Centron D, Vay C, *et al.* IS CR2 and IS 26: Two insertion sequences highly dispersed among Acinetobacter spp. clinical strains. J Bacteriol Mycol Open Access 2017;4:33-6.
11. Campbell NA, Reece JB. Biology. 6th ed. San Francisco: Benjamin Cummings; 2002. p. 345-6.
12. Pagano M, Martins AF, Barth AL. Mobile genetic elements related to carbapenem resistance in *Acinetobacter baumannii*. Braz J Microbiol 2016;47:785-92.
13. Forbes BA, Sahm DF, Weissfeld AS. Diagnostic Microbiology. St Louis: Mosby; 2007.
14. Brooks GF, Carroll KC, Butel JS, Morse SA, Mietzner TA. Medical Microbiology. Jawetz, Melnick and adelberg's. 26th ed. USA: McGraw-Hill Companies, Inc.; 2013.
15. Ling TK, Liu ZK, Cheng AF. Evaluation of the VITEK 2 system for rapid direct identification and susceptibility testing of gram-negative bacilli from positive blood cultures. J Clin Microbiol 2003;41:4705-7.
16. CLSI. CLSI Supplement M100. Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.
17. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, *et al.* Multidrug-resistant, extensively drug-resistant and

[Downloaded free from http://www.mmjonweb.org on Monday, July 4, 2022, IP: 37.237.24.42]

pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268-81.
18. 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;11:1249-60.
19. Higgins PG, Dammhayn C, Hackel M, Seifert H. Global spread of carbapenem-resistant *Acinetobacter baumannii*. J Antimicrob Chemother 2010;65:233-8.
20. Al-Saadi FS. 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.
21. Hussein NH, Al-Mathkhury HJ, Sabbah MA. Imipenem-Resist ant *Acinetobacter baumannii* isolated from patients and hospitals environment in Baghdad. Iraqi J Sci 2013;54:803-12.
22. Salman SB, Al-Mathkhury HJ. Molecular detection of *Klebsiella pneumoniae* serotype K2 isolated clinically. Iraqi J Sci 2016;57:98-103.
23. 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:E58251.
24. Evans BA, Hamouda A, Towner KJ, Amyes SG. OXA-51-like beta-lactamases and their association with particular epidemic lineages of *Acinetobacter baumannii*. Clin Microbiol Infect 2008;14:268-75.
25. Anil C, Nirav P. Emergence of multidrug resistant *Acinetobacter baumannii* as nosocomial pathogen: Clinical significance and antimicrobial sensitivity. IOSR 2015;14:853-2279.
26. Zavascki AP, Carvalhaes CG, Picão RC, Gales AC. Multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: Resistance mechanisms and implications for therapy. Expert Rev Anti Infect Ther 2010;8:71-93.
27. Sohrabi B, Vanani IR, Tahmasebipur K, Fazli S. An exploratory analysis of hotel selection factors: A comprehensive survey of Tehran hotels. Int J Hosp Manag 2012;31:96-106.
28. 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;27:351-3.
29. Ghaima KK, Saadedin SM, Jassim KA. Isolation, molecular identification and antimicrobial susceptibility of *Acinetobacter baumannii* isolated from Baghdad. Int J Sci Res Publ 2016;6:351-6.
30. Abouelfetouh A, Torky AS, Aboulmagd E. Phenotypic and genotypic characterization of carbapenem-resistant *Acinetobacter baumannii* isolates from Egypt. Antimicrob Resist Infect Control 2019;8:185.