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Whole-genome Study of Carbapenem-resistant *Acinetobacter baumannii* Virulence and Resistance

Rasha H. Shayea*, Munim R. Ali

Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq

ABSTRACT

Background and Aim: Carbapenem-resistant *Acinetobacter baumannii* (CRAb) has the ability to develop and acquire resistance making it one of the most critical nosocomial pathogens globally. Whole genome sequencing (WGS) technology was employed to map genes associated with antimicrobial resistance (AMR), and virulence factors and to identify multilocus sequence types (MLST). In order to understand the resistance mechanism for *A. baumannii* species, this study set out to establish the genetic makeup of the species.

Materials and Methods: Whole-genome sequencing (WGS) of *A.b*4, *A.b49,* and *A.b*75 was performed using Illumina MiSeq and the genomes were assembled with SPAdes. ARG-ANNOT, CARD-RGI, VFDB, PHAST, PlasmidFinder were used to analyze all genomes.

Results: Genome analysis revealed that Ab4 belongs to ST944, represented singletons that could not be attributed to any, A.b49 belongs to ST1104, representing unique ST.While A.b75 belongs to ST195 which represented known international clones of high risk. Molecular characterization showed the presence 23 antibiotic-resistance genes in all strains of *A. baumannii*. 12 of them are shared by all 3 strains and 11 are common between *A. baumannii* 4(ST/944), 49 (ST/1104), and 75(ST/195). The common drug-resistance genes shared by all 3 strains include *bla* $_{OXA-72}$ (resistance to carbapenems), *ade* genes, RND (*adeFJK*, *adeLN* & *adeR*), and SMR (*abeS*) encoding for efflux pumps.

Conclusion: We present WGS analysis of three *A. baumannii* strains belonging to three different STs. The presence of strains harboring acquired AMR genes makes them more dangerous. Acquired resistance genes and chromosomal gene mutation are successful routes for disseminating AMR determinants among *A. baumannii*. Identification of chromosomal and plasmid-encoded AMR in the genome of *A. baumannii* may help understand the mechanism behind the genetic mobilization and spread of AMR genes.

Keywords: *A. baumannii*, Antibiotic resistance, Carbapenem-resistant, Multi-Locus Sequence Typing (MLST), Virulence traits, WGS

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Corresponding Information: **Rasha H. Shayea**, *Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq*
Email: rasha.jk.bio@gmail.com

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1. Introduction

The most common cause of extensively drug-resistant (XDR) and multidrug-resistant (MDR) nosocomial infections worldwide is the well-known Gram-negative bacterium *Acinetobacter baumannii* **(1, 2)**. In hospital settings worldwide, the rise of MDR *A. baumannii* strains that are resistant to last-resort antibiotics like carbapenems and colistin is complicating therapy **(3)**.

Reduced membrane permeability, decreased efflux pump activity, and the production of a variety of ß-lactamase enzymes are all ways that *A.*

baumannii might gain intrinsic resistance **(4)**. However, this pathogen's resistance is commonly linked to mobile genetic elements (MGEs) that can be passed from one bacterial species to another, allowing for the quick spread and maintenance of resistance genes amongst other bacterial species **(5)**. In addition to certain various naturally occurring intrinsic resistance genes, it can also acquire resistance through mutational alterations in chromosomal structure and horizontal gene transfer **(6, 7)**. Whole-genome sequencing (WGS) has recently enabled resolution in highly related lineages without the requirement for species-dependent protocols, in addition to providing information on pathogen identification, virulence factors, drug susceptibility, comparative genomics, and the detection and investigation of outbreaks **(8, 9)**. Additionally, Comparative genomic analysis of *A. baumannii* strains revealed that the genome of *A. baumannii* could acquire a large amount of foreign DNA, which could play a role in antimicrobial resistance and pathogenesis **(10)**. In order to understand the resistance mechanism for *A. baumannii* species, this study set out to establish the genetic makeup of the species.

2. Materials and Methods

Identification of Bacterial Isolates

A total of 75 isolates have been taken from various hospitals in Baghdad (Central Children's Hospital, Baghdad Teaching Hospital, Burn Hospital, Children Protection Teaching Hospital (Medical City), Al-Imameen Al-Kadhimayn City Hospital) during the three months from March 2021 to June 2021. The isolates included: 75 specimens taken from wounds, urine, respiratory infections (sputum), burns, and CSF in patients setting in hospitals, particularly in intensive care units. All the isolates were characterized via VITEK 2 System, the biochemical reactions between the bacterial isolates that have been suspended in their solutions, and media in VITEK2 Identification Cards. The specimens were transported to the laboratory (via transport media) and cultured on *A. baumannii*-selective media (Chrom agar) and MacConkey agar, incubated at a temperature of 37ºC for 24hrs under aerobic conditions. Then the colony's color, shape, edges, and texture were examined **(11)**. Antimicrobial susceptibility testing was performed using a disk diffusion method, against sixteen different antibiotics, the results were interpreted using the Clinical and Laboratory Standards Institute (CLSI) categories **(12)**. Bacterial DNA was extracted from the Nutrient agar after 24 hours of incubation at 37ºC according to the boiling method as Annotated in Abed & Ali **(13)**. Genotyping detection was confirmed by amplifying the *blaOXA-51* gene for *A. baumannii* confirmation, detection by PCR technique in comparison with positive controls strain **(14)**. Tri-locus multiplex PCR as described by Turton *et al*. **(15)**, was used to determine clonal relationships of these *A. baumannii* clinical isolates [Global Clones (GC) I, II, or III, also known as International Clones I, II, or III]. The isolate was fingerprinted by ERIC-PCR based on the methods of Coudron *et al.* **(16)**.

Whole-Genome Sequencing and Collection of Sequence Data

Three *A. baumannii* (4,49 & 75) isolates were available for WGS. DNA extraction was performed using the High-Pure template preparation kit (Roche Applied Sciences, Mannheim, Germany) according to the manufacturer's instructions. The library preparation and paired-end sequencing on an Illumina MiSeq sequencer were performed for the three *A. baumannii* strains as previously described **(4)**. Briefly, the Illumina TruSeq Nano DNA kit (Illumina, Inc., San Diego, CA, USA) was utilized to prepare the sequencing library, followed by paired-end sequencing on an Illumina MiSeq sequencer (Illumina, San Diego, CA, USA).

The results were filtered, and the search items following the Library Layout (paired), Library Source (genomic), and Platform (Illumina) were eligible for inclusion.

Multilocus Sequence Typing.

The sequences for the Institut Pasteur MLST genes, were extracted from the assembled contigs for all isolates, concatenated, and aligned with MUSCLE. A phylogeny was inferred with MEGA version 5.2.2, using the maximum-likelihood method, and exported to FigTree version 1.4.2 for visualization. In addition, sequence types were determined for all *A. baumannii* isolates using the database available on the Institut Pasteur MLST website (http://www.pasteur.fr/mlst).

Bioinformatics Data Analysis

Data analysis of sequences from three isolates of *A. baumannii* sequenced within this study and downloaded sequences was performed with the pipeline WGSBAC (v2.0.0) **(4, 17)**. In short, raw sequencing data quality was controlled by WGSBAC with FastQC (v. 0.11.5), and coverage was determined **(18)**. Shovel (v. 1.0.4), based on SPAdes (v3.14.0), was used for assembly **(19)**. Assembly quality was checked with QUAST (v. 5.0.2) **(20)**. Kraken 2 (v. 2.0.7 beta), in combination with the database MiniKraken (v2), was used to classify reads and assemblies and to check for contamination **(21)**. The assembled genome sequence was annotated by the NCBI Prokaryotic Genome Automatic Annotation

Pipeline (PGAAP) which uses AMR Finder Plus, ResFinder, and CARD For antimicrobial resistance profiling and determination of AMR genes **(22-24)**. The VFDB database was used to detect the virulence traits **(25)**. tRNAscan-SE for tRNA genes **(26)** , and RNAmmer for rRNA genes **(27)**. The regions with abnormal G+C contents in the genomic sequence were obtained by using the GC-Profile program (28). Prophage regions and CRISPR-Cas proteins were detected using the web tools, PHASTER **(29)** and CRISPRCasFinder **(30)**, respectively. PlasmidFinder and Platon were used to investigate the potential presence of plasmids and plasmid replicons **(31, 32)**.

Data Access

This sequences data of this study has been submitted to the NCBI BioProject (http:// www.ncbi.nlm.nih.gov/bioproject) under BioProject accessions:

PRJNA850024, PRJNA802181, and PRJNA850033, Respectively.

3. Results

During the study period, A total of 75 isolates of *A. baumannii* recovered from clinical samples. Isolates were confirmed as *A. baumannii* by the presence of intrinsic, *bla* $_{OXA-51}$-like gene (Figure1). Most of the *A. baumannii* isolates were obtained from the ICU (40%). Bacterial isolates were predominantly recovered from lower respiratory tract specimens (*n* = 20/75; 26%), blood samples (*n* = 18/75; 24%), burn swaps (*n* = 13/75; 17%), wound exudates (*n* = 10/75; 13%), urine samples (*n* = 7/75; 9%), CSF samples (*n* = 4/75; 5%), and fluid specimens (*n* = 3/75; 4%).

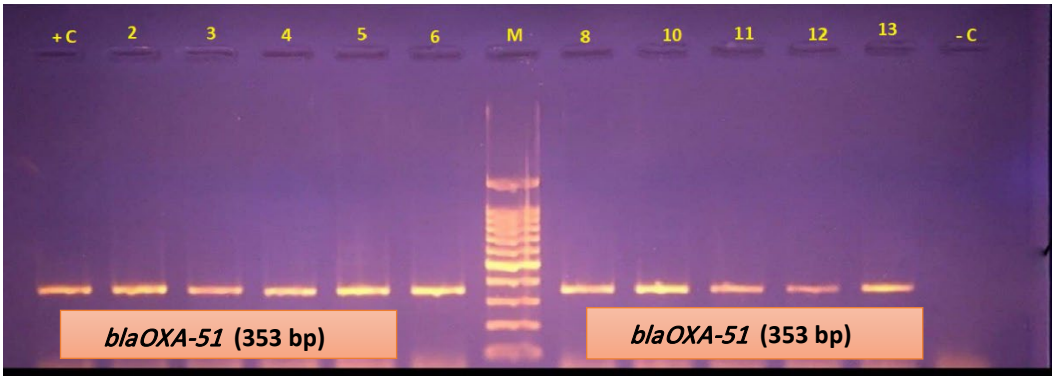

Figure 1. The blaOXA-51 gene product size (band 353 bp) was detected by using agarose gel electrophoresis (1% agarose, 7v/cm2) with Ethidium bromide staining. Utilizing the template DNA that was prepared with the use of the boiling method. The molecular size of the DNA ladder was 100 bp, located in the middle (M). DNA isolated from *A. baumannii* samples have shown positive PCR, with a positive control on the right and negative control on the left.

Based on the results of the categories of resistance, Genotyping detection by using ERIC-PCR typing and Epidemiological typing represented by Global lineage, we selected 3 isolates, based on the variety of outcomes within each chosen category, the isolates were chosen (Table 1).

Table 1. Criteria used to select isolates for WGS

Character	*A.baumannii* (4)	*A.baumanni* (49)	*A.baumannii* (75)
Source	Blood	Urine	Blood
Gender	Female	Male	Female
Resistance categories	6/16 (MDR)	16/16(PDR)	15/16 (XDR)
Global lineage	G2	G3	G1
ERIC-PCR (Band no. and size)	Group E 1 band (550bp)	Untypable	St (out of group) 1 band (580bp)

In this study, we tried to capture as much diversity of *A. baumannii* clones as feasible while keeping the number of isolates as low as possible for clarity of presentation. Selected Three isolates, AB75 assigned: ST195 which represented known international clones of high risk and AB49 assigned: ST1104 represented unique ST ,while AB4 isolate represented singletons that could not be attributed to any ICs, possessed a novel sequence type that was not clustered with

existing international clones by either cgMLST or MLST loci (Figure 2).

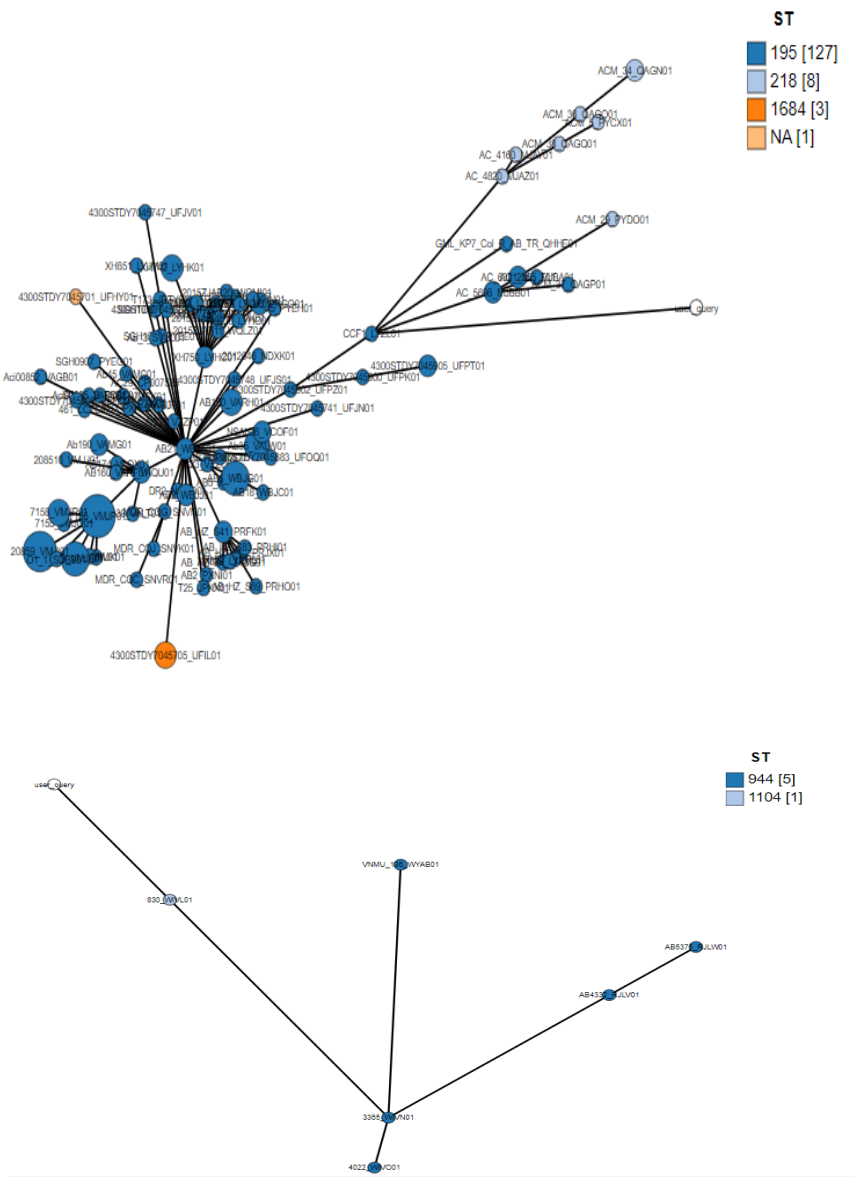

Figure 2. Based on cgMLST characteristics, the minimal spanning tree for the isolates examined (4,49 and 75). The numbers reflect amount of distinct alleles found in the pairs of related isolates. Close isolates are shown in the same color; other isolates are not.

Whole Genome Sequencing of the Three *A. baumannii* Strains

After de novo assembly and manual gap-closing by PCR and re-sequencing using the Sanger sequencing method, the complete genomes of *A. baumannii* 4 (ST/944), *A. baumannii* 49 (ST/1104), and *A. baumannii* 75 (ST/195) strains yield 3,926741 bp, 9,241711 bp, and 3,995107 bp with a G+C content of 38.74%, 50.61%, and 38.96%, respectively. The number of contigs of *A.baumannii* 4(ST/944) , 49(ST/1104), and 75(ST/195) was 34, 165, and 89. The mean N50 of the three assembled genomes was 283689, 231932, and 121908, respectively (Table 2 and Figure 2). The characteristics of the three genomes are listed in Table 2. Using the NCBI Prokaryotic Genome Automatic Annotation Pipeline (PGAAP) and the genome of *Acinetobacter baumannii* strain GU71 (470.4681) as a reference sequence, we predicted 3,761, 9,251, and 3909 protein-coding sequences from *A. baumanni* 4(ST/944), *A. baumannii* 49(ST/1104), and *A. baumannii* 75(ST/195) genomes, respectively. The complete ribosomal cluster was 64 tRNAs were identified in the genomes of each *A. baumannii* 4 (ST/944) and 75(ST/195), while 144 tRNAs were identified for *A. baumannii* 49(ST/1104). Additionally, rRNAs were 3 for each of *A. baumannii* 4(ST/944) & 75(ST/195), while 5 rRNAs were for *A. baumannii* 49(ST/1104).

Table 2. Genome features of *A. baumannii* 4,49 & 75 strains

Characteristic	*A. baumannii* 4	*A. baumannii 49*	*A. baumannii* 75
Genome ID	470.14854	470.14818	470.14828
Genome Length	3926741 bp	9241711 bp	3995107 bp
G+C content (%)	38.74	50.614902	38.96
Number of contigs	34	165	89
Contig L50	3	10	11
Contig N50	283689	231932	121908
Coarse Consistency	99.4	97.7	99.5
Fine Consistency	98.7	59.6	99
Genome Quality	Good	Poor	Good
CDS	3761	9251	3909
tRNA	64	144	64
rRNA	3	5	3
Transporter	41	299	44
Drug Target	10	174	11

CRISPR-Cas System

A subtype I-F CRISPR-Cas was identifed on the chromosome of *A. baumannii* 4(ST/944) only, with a 2 bp CRISPR array containing 114 spacers. The spacers are flanked by 116 bp of CRISPR repeats. It indicated that strains with close phylogenetic relationships have similar genes and share gene synteny on their CRISPR-Cas system.

Prevalence of Antimicrobial Resistance Genes

The *in-silico* detection of acquired AMR genes in *A. baumannii* 4 (ST/944),49 (ST/1104) and 75(ST/195) isolates based on WGS data using the ResFinder database succeeded in identifying 51 acquired AMR genes (Supplementary Table 3). Among the 23 drug-resistance-related genes, 12 of them are shared by all 3 strains and 11 are common between *A. baumannii* 4(ST/944),49 (ST/1104) and 75(ST/195). The common drug-resistance genes shared by all 3 strains include *bla* $_{\text{OXA-72}}$ (resistance to carbapenems), *ade* genes, RND (*adeFJK*, *adeLN* & *adeR*), and SMR (*abeS*) encoding for efflux pumps. *ANT(3'')-IIc* and *A.baumannii AbaF* (aminoglycoside resistance), *A.baumannii parC,* and *A.baumannii AbaQ* responsible for resistance to fluoroquinolones were also identified in all 3 *A. baumannii* strains.

A. baumannii 49(ST/1104) and *A. baumannii* 75(ST/195) shared all drug-resistant genes except *blaOXA66*, ade(AG), and *ADC-73* (Rrisitance to cephalosporin), which are unique to *A.baumannii* 75(ST/195), while *blaOXA90* and *ADC-52* genes, which are unique to *A.baumannii* 49(ST/1104). *A. baumannii* 4 (ST/944) harbors 15 drug-resistance genes, 12 of which are shared with the other two stains and three of which are unique to *A. baumannii*4, including *bla OXA99, adeG*, and *ADC-154.*

Prediction of virulence genes

The VFDB database **(25)** was used to characterize the virulence traits present in *A.baumannii* 4(ST/944) ,49 (ST/1104), and 75(ST/195). A total of 54 virulence-related genes were determined in the genomes of *A.baumannii* 4(ST/944), 49(ST/1104), and 75(ST/195) (Supplementary Table 4). Most of these genes are involved in efflux pumps, capsular polysaccharide lipopolysaccharide, biofilm formation, quorum sensing, outer membrane protein ompA, a two-component regulatory system, iron uptake, phospholipase C and D, serum resistance, and some putative virulence factors. These genes were shared by three strains of *A. baumannii* 4(ST/944), 49(ST/1104), and 75(ST/195) (See Table 4). Most of the genes involved in pathogenesis are the same as those in other *A. baumannii* isolates. Except for the (*pilE*) genes, which encode for Type IV pili, are unique to the *A. baumannii* 4 (ST/944) strain. Additionally, the pathogenesis genes (*fliM* and *rcsAB*) are unique to strain *A. baumannii* 49(ST/1104) but not found in other strains.

Table 3. List of acquired antimicrobial resistance genes identified in *A. baumannii* (4,49 and 75) isolates based on WGS data using ResFinder databases

AMR gene	Mechanism	Frequency in three isolates	Predicted phenotype
A.baumannii AbaF	Antibiotic efflux	3	fosfomycin
A.baumannii AbaQ	antibiotic efflux	3	fluoroquinolone antibiotic
A.baumannii gyrA	antibiotic target alteration	2	
A.baumannii parC	antibiotic target alteration	3	
abeS	Antibiotic efflux	3	Macrolide antibiotic aminocoumarin antibiotic
ADC-152	antibiotic inactivation	1	Cephalosporins
ADC-154	antibiotic inactivation	1	
ADC-73	antibiotic inactivation	1	
adeA	Antibiotic efflux	1	Glycylcycline antibiotic tetracycline antibiotic
adeF	Antibiotic efflux	3	Fluoroquinolone antibiotic tetracycline antibiotics
adeG	Antibiotic efflux	2	
adeH	Antibiotic efflux	2	
adeI	Antibiotic efflux	2	macrolide antibiotic fluoroquinolone antibiotic lincosamide antibiotic carbapenem cephalosporin tetracycline antibiotic rifamycin antibiotic diaminopyrimidine antibiotic phenicol antibiotic penem
adeJ	Antibiotic efflux	3	
adeK	Antibiotic efflux	3	
adeL	Antibiotic efflux	3	Fluoroquinolone antibiotic tetracycline antibiotics
adeN	Antibiotic efflux	3	macrolide antibiotic fluoroquinolone antibiotic lincosamide antibiotic carbapenem cephalosporin tetracycline antibiotic rifamycin antibiotic diaminopyrimidine antibiotic phenicol antibiotic penem
adeR	Antibiotic efflux	3	Glycylcycline antibiotic tetracycline antibiotic
ANT(3'')-IIc	Antibiotic inactivation	3	Aminoglycosides
OXA-66	antibiotic inactivation	1	Carbapenem Cephalosporin penam
OXA-72	antibiotic inactivation	3	
OXA-90	antibiotic inactivation	1	
OXA-99	antibiotic inactivation	1	

Table 4. The virulence genes in *A. baumannii*4,49 and 75 strains

VF Class	Virulence factors	Genes	*A. baumannii*4 No. of genes	*A.baumannii* 49 No. of genes	*A. baumannii* 75 No. of genes
Adherence	Outer membrane protein	*ompA*	+ (1)	+ (1)	+ (1)
	Type IV pili	*pilE*	+ (1)	-	-
	AdeFGH Efflux pump/transport autoinducer	*AdeFGH*	+ (3)	+ (3)	+ (3)
Biofilm formation	Biofilm-associated protein	*bap*	+ (1)	+ (1)	+ (1)
	Csu pili	*csuA/B, csuA, csuB, csuC, csuD, csuE*	+ (6)	+ (6)	+ (6)
	PNAG (Polysaccharide poly-N-acetylglucosamine)	*pgaA. pgaB, pgaC, pgaD*	+ (4)	+ (4)	+ (4)
Motility	flagellar motor switch protein FliM	*fliM*	-	+ (1)	-
Enzyme	Phospholipase C	*Plc, plc,*	+ (1)	+ (2)	+ (1)
	Phospholipase D	*plcD*	+ (1)	+ (1)	+ (1)
Immune evasion	Capsule	*mynA/sacA, rmlC, wbjD/wecB,gnd, probable wbaZ, wzb*	-	+ (8)	-
	LPS	*lpsB, lpxA, lpxB, lpxC, lpxD, lpxL, lpxM, acpXL*	+ (7)	+ (8)	+ (7)
Iron uptake	Acinetobactin	*barA, barB, basA, basB, basC, basD, basF,basG, basH, basI, basJ, bauA, bauB, bauC, bauD, bauE, bauF, entE*	+ (18)	+ (18)	+ (18)
	Heme utilization	*hemO*	+ (1)	+(1)	+ (1)
Regulation	Quorum sensing	*abaI, abaR*	+ (2)	+ (2)	+ (2)
	Two-component system	*bfmR, bfmS*	+ (2)	+ (2)	+ (2)
	transcriptional regulator	*rcsA,B*	-	+ (2)	-
Serum resistance	pbpG	*pbpG*	+ (1)	+ (1)	+ (1)
tress adaptation	Catalase (Neisseria)	*KatA, katG*	+ (1)	+ (2)	+ (1)

In Silico Detection of Plasmids

We also used PlasmidFinder and plasmidSPAdes software to search for plasmids or plasmid-related sequences in genomes of *A.baumannii* 4(ST/944),49(ST/1104) and 75(ST/195). One conjugative plasmid (pWP8-W18-ESBL-11) was found in *A.baumannii* 4(ST/944), and three plasmids (pVB473_1, pAbCTX11 and ABAY15001_6E) were found in *A. baumannii* 49(ST/1104). While two plasmids(pVB473_1 and pABAY15001_6E) were identified in *A. baumannii* 75(ST/195).

Prophage Regions

Prophage sequences were identified using the PHASTER server **(29)**. In *A. baumannii* 4(ST/944), three prophage regions have been identified, two of which are intact and one region is incomplete. Both *A.baumannii* 49(ST/1104) and *A.baumannii* 75(ST/195) strains have 9 prophage regions, two regions are intact and seven are incomplete for each strain (Table 5).

The intact prophage regions of all strains may have (tail, integrase, portal, capsid, lysis, terminase, head, recombinase & plate or tail, head & terminase or tail, capsid, terminase). While incomplete prophage regions, there may be (plate& transposase or tail & integrase or head& capsid or only N.A. or integrase or tail& recombinase).

Table 5. prophage identification in *A. baumannii* 4,49 and 75 strains

Character	*A.baumanni* 4		*A.baumannii* 49		*A.baumannii* 75	
	Region	Most common phage	Region	Most common phage	Region	Most common phage
Intact	5	PHAGE_Acinet_Bphi_B1251_NC_019541, ...	1	PHAGE_Edward_GF_2_NC_026611(18)	2	PHAGE_Acinet_vB_AbaS_TRS1_NC_031098(8)
	10	PHAGE_Salmon_SSU5-NC- 018843(23)	2	PHAGE_Erwini_EtG_NC_047833(8)	14	PHAGE_Mannhe_vB_MhM_3927AP2_NC_028766(12)
	10	PHAGE-Pseudo-nickie-NC- 042091(16)	1	PHAGE_Pectob_ZF40_NC_019522(13)	12	PHAGE_Acinet_YMC11/11/R3177_NC_041866(3)
incomplete			9	PHAGE_Escher_500465_1_NC_049342(8)	26	PHAGE_Acinet_vB_AbaS_TRS1_NC_031098(8)
			20	PHAGE_Paenib_Tripp_NC_028930(2)	34	PHAGE_Acinet_Bphi_B1251_NC_019541(9)
			33	PHAGE_Acinet_Bphi_B1251_NC_019541(9)	39	PHAGE_Acinet_Bphi_B1251_NC_019541(18)
			47	PHAGE_Acinet_Bphi_B1251_NC_019541(5)	42	PHAGE_Acinet_YMC11/11/R3177_NC_041866(18)
			49	PHAGE_Acinet_Bphi_B1251_NC_019541(12)	46	PHAGE_Psychr_pOW20_A_NC_020841(6)
			67	PHAGE_Phage_Gifsy_1_NC_010392(4)	58	PHAGE_Acinet_Bphi_B1251_NC_019541(8)

4. Discussion

The genus Acinetobacter represents an important group of pathogens, mainly due to its extreme genome plasticity and the ability to acquire foreign DNA **(33)**. To analyze the multidrug resistance phenotype of *A. baumannii* strains 4(ST/944), 49(ST/1104), and 75(ST/195) on a molecular basis, their whole genomes were sequenced to identify antibiotic resistance and virulence genes to determine their relationship to other sequenced clinical and multiresistant *A. baumannii* isolates, also considering epidemiological aspects.

MLST analysis confirmed the considerable genetic diversity of *A. baumannii* in the investigated strains from Baghdad hospitlization. MLST analyses based on the Pasteur scheme identified 195 sequence types (ST) for *A.baumannii* 75 strain was the most prevalent sequence type circulating in the Baghdad hospitals. ST/195 belongs to the international clones and is the most dominant type globally **(34)**. In previous studies, most of the CRAb isolates were found belong to the ST2 lineage in strains isolated from Thailand , Myanmar and among strains isolated from other countries **(35, 36)**. *A. baumanniia* 49 strain that assigned: ST1104 represented unique ST ,could not be assigned to a distinct sequence type due to new alleles. While *A. baumannii* 4 strain that contain 944 sequence types (ST) were unique singletons, possessed a novel sequence type that was not clustered with existing international clones by either cgMLST or MLST loci . These findings are, moreover, in agreement with those of Gaiarsa *et al*., who demonstrated that the Pasteur scheme is more appropriate for epidemiological studies of *A. baumannii* and proposed it to be the scheme of choice in parallel with cgMLST **(37)**.

As a rule of thumb, 5–10X coverage is suggested to support sequence assembly and genome reconstruction **(38)**. In our study, only one isolate of *A. baumannii* **(39)** had a sequencing depth below 5X (poor quality). One reasing for low sequencing depth is can be influenced by errors at many stages during DNA processing and library preparation, such as amplification errors, DNA quality, and target region complexity **(40, 41)**. Another reason for low sequencing depth could be that the Illumina sequencing platform favors GC-balanced regions that have fewer reads in GC-poor regions, resulting in unequal sequencing depth across the genome **(42)**. In our study, GC content in *A. baumannii* 4(ST/944) (38.7 %), *A. baumannii* 75(ST/195) (38.9 %) with good quality, and *A. baumannii* 49(ST/1104) (50.6 %) with low quality does not agree with the statement above. But agree with Kauser **(43)**, were found that the average GC percent in *E. coli* (50 %), *K. pneumoniae* (56%) with low quality, and *A. baumannii* (39%) with high quality. Regarding contiguity, 4(ST/944), 49(ST/1104), and 75(ST/195) isolates of *A. baumannii* had 34, 165, and 84 contigs, respectively, with a high degree of completeness (100%) from the BUSCO score, indicating successful genome assembly and accurate analysis.

The assembly of the *A.baumannii* 4(ST/944),49(ST/1104) and 75(ST/195) strains yielded 3926741 bp, 9241711 bp and 395107 bp with an N50 contig size of 283689, 231932 and 121908 bp and a G+C content of 38.74%, 50.614902% and 38.96%,

respectively (Table 2). To check for possible contamination and accurate species identification, the software Kraken2 was used, which classifies each read (or contig) **(19, 44)**. The number of predicted proteins, as well as the average length of the genes, is comparable with other previously sequenced genomes (*Acinetobacter baumannii* strain GU71 470.4681 as a reference sequence). At the species level, the first match for all *A. baumannii* strains belongs to the *A. baumannii* species. Furthermore, the phylogenetic position of each strain among available Acinetobacter genomes was studied based on a previously described pipeline **(45)**.

RISPR-Cas was identified on the chromosome of *A. baumannii* 4(ST/944) only. Since the spacers in the CRISPR array do not change over time, the high number of spacers identified in the CRISPR loci of this pathogen suggests that it might have encountered a high number of phage attacks. The presence of the CRISPR-Cas system could explain the low number of plasmids found within this genome **(46)**.

Antimicrobial susceptibility testing showed that the 3 *A.baumannii* strains are resistant to almost all commonly used antibiotics. The genetic variations responsible for resistance to most the antibiotics have been identified in all *A. baumannii* strains (Table 3). It is well known that the acquisition of genetic determinants in *A. baumannii* is crucial to the evolution of this pathogen. A combination of different mechanisms such as transformation, conjugation, and transduction and a variety of elements play a role in this process. Key elements directly involved in the acquisition of genetic material are ISs and transposons, genomic islands, integrons, and plasmids **(44, 47)**. Genes involved in resistance to, fluoroquinolone, macrolide, aminoglycoside, and β-lactam were found in the *A. baumannii* 4(ST/944), 49(ST/1104), and 75(ST/195) genomes.

Resistance to β-Lactams: In total, nine AMR genes mediating resistance to ß-lactams, including cephalosporins and carbapenems, were identified. The Ambler class D β-lactamases were present in almost all strains. The variants of the intrinsic $bla_{\text{OXA-72}}$ carbapenemase gene were the most frequent and was found in three *A. baumannii* strains; $bla_{\text{OXA-99}}$ was found in *A. baumannii* 4(ST/944) isolate, followed by $bla_{\text{OXA-90}}$ was found in *A. baumannii* 49(ST/1104) isolate and $bla_{\text{OXA-66}}$ was present in *A. baumannii* 75(ST/195) isolate. Three *Acinetobacter*-derived cephalosporins bla_{ADC} variants of the Ambler class C β-lactamases were identified. The $bla_{\text{ADC-154}}$ was detected in *A. baumannii* 4(ST/944) isolate, followed by $bla_{\text{ADC-152}}$ in *A. baumannii* 49(ST/1104) isolate and $bla_{\text{ADC-73}}$ in *A. baumannii* 75(ST/195) isolate (Table 3).

Two ambler classes of β-lactamases (i.e., classes C and D) were identified in the current study. Various resistance genes conferring resistance to carbapenems and cephalosporins were found in *A. baumannii* isolated from hospitals in Baghdad. The $bla_{\text{OXA-72}}$ variants were among the most frequent AMR genes identified. Which are currently spreading on plasmids and associated with resistance to all β-lactam compounds, including carbapenems **(48, 49)**. The ADC beta-lactamases are cephalosporinases with extended-spectrum resistance to cephalosporins. All strains harbored bla_{ADC} gene, which are considered significant determinant responsible for cephalosporins resistance in *A. baumannii* **(39)**.

Resistance to Aminoglycosides: In the three analyzed strains, 3 AMR genes conferring resistance to aminoglycosides were identified. Aminoglycoside-modifying enzymes (AMEs), including nucleotidyltransferases (ANTs), were identified in all strains of *A. baumannii*. Aminoglycosides are broad-spectrum antibiotics used against a wide range of infections caused by Gram-negative bacteria in clinical settings. However, their efficacy has been reduced by resistance development **(50)**. This finding highlights the diversity of aminoglycoside-resistant *A. baumannii* strains mediated by ANT (3'')-IIc in Baghdad hospitals.

The fluoroquinolone resistance in *A. baumannii* 4(ST/944), 49 (ST/1104) and 75(ST/195) correlated with the results of Ostrer *et al*., which stated that it could be predicted based solely on target gene quinolone-resistance mutations for *A. baumannii* and that the primary mutation is followed by either of two mutations in the alternate target in this species, ParC88 ← GyrA81 → ParC84 **(51)**.

Antibiotic Efflux Pumps: Four categories of efflux pumps were found in *A. baumannii* isolates, including the resistance-nodulation-division (RND) superfamily, the major facilitator superfamily (MFS), the multidrug and toxic compound extrusion (MATE) family and the small multidrug resistance (SMR) family transporters. Among these different pumps, the RND (*adeFJK*, *adeLN* & *adeR*), and SMR (*abeS*) were most frequent and found in almost all isolates. RND efflux pump-coding genes (*adeG*) were found in *A. baumannii* 4(ST/944) and 75(ST/195) strains, (*adeIH)* were found in *A.baumannii* 49(ST/1104) and 75(ST/195) strains, while (*adeA)* were found only in *A. baumannii* 75(ST/195) strain (Table 3). Resistance mediated by antibiotic efflux pump-encoding genes is well documented in *A. baumannii* **(52)**. Efflux pumps play significant roles in developing AMR in *A. baumannii* **(53)**. The circulation of numerous efflux pumps with high frequency suggests a significant rise in *A. baumannii* antibiotic resistance in Iraq. *Acinetobacter baumannii Aba*F was found in all

strains of *Abaumannii* isolated from Baghdad hospitals. *Aba*F is a major facilitator superfamily (MFS) antibiotic efflux pump interfering with protein synthesis. Its expression in *A.baumannii* increases resistance to fosfomycin. The high frequency of resistance to fosfomycin was seen in the tested strains and in a previous study on *A. baumannii* **(4)**. It has been reported that the *aba*F gene is involved in fosfomycin resistance in *A. baumannii* and plays a role in biofilm formation and virulence mechanisms **(54)**.

A total of 18 virulence factors with 54 virulence-related genes were determined in the genomes of *A.baumannii* 4(ST/944), 49(ST/1104) and75 (ST/195) (Table 4). Analysis of the genes showed that most of these genes and their role in virulence were shared by all strains of *A.baumannii* genomes. It appeared that *A. baumannii* strains possess different virulence genes featuring predicted functions in adherence, biofilm formation, immune evasion, iron uptake, serum resistance, and motility, among others (Table 4), which facilitate their survival under unfavorable conditions **(55)**. Also, outer membrane protein ompA was found in all strains of *A. baumannii*, which induces cytotoxicity and constitutes a virulence factor with multiple important effects on the pathogenesis of *A. baumannii* **(56)**. Another important virulence factors identified in *A. baumannii* are LPS. It has been play a role in pathogenesis, allowing the bacteria to resist the bactericidal activity of complement and providing protection from diverse environmental conditions such as desiccation and antibiotic exposure **(57)**.

Acinetobacter baumannii 4(ST/944), 49(ST/1104) and 75(ST/195) isolates contain one, three, and two conjugative plasmids respectively. Plasmids in our isolates were linked to pathogen virulence and the formation of drug-resistant persister cells and biofilms **(58)**.

In addition, using the PHAST tool to predict phage sequences, It has been documented that the presence and/or the number of prophages are not homogeneous in bacterial genomes, possibly due to their intrinsic genetic variability **(59, 60)**. This was observed in our work where we found different amounts of prophages in the genomes analyzed (Table 5). Moreover, it is likely that the presence of a CRISPR-cas system in *A. baumannii* 4(ST/944) had contributed to the absence of more prophages.

5. Conclusion

We present WGS analysis of three *A. baumannii* strains belonging to three different STs. The presence of strains harboring acquired AMR genes makes them more dangerous. Acquired resistance genes and chromosomal gene mutation are successful routes for disseminating AMR determinants among *A. baumannii*. Identification of chromosomal and plasmid-encoded AMR in the genome of *A. baumannii* may help understand the mechanism behind the genetic mobilization and spread of AMR genes. WGS technologies and bioinformatics analysis tools for nosocomial outbreak analyses allow the resolution of highly related lineages without the need for species-dependent protocols. Moreover, it gives information on pathogen identification, virulence factors, drug susceptibility, comparative genomics, and detection and investigation of outbreaks in recent years.

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Conflict of interest

The authors declare that there is no conflict of interest in the publication.

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