Journal of Communicable Diseases
Volume 56, Issue 1 - 2024, Pg. No. 106-119
Peer Reviewed & Open Access Journal

Research Article

Molecular Analysis of Whole Genomic Sequencing for *Acinetobacter Baumannii* Isolates under Antibiotic Stress in a Hospital Outbreak

Esraa S Abed[1], Munim R Ali[2]

[1,2]College of Dentistry, [2]College of Science, Mustansiriyah University, Baghdad, Iraq.
DOI: https://doi.org/10.24321/0019.5138.202415

I N F O

Corresponding Author:
Esraa S Abed, College of Dentistry, 2College of Science, Mustansiriyah University, Baghdad, Iraq.
E-mail Id:
essrabio995@uomustansiriyah.edu.iq
Orcid Id:
https://orcid.org/0000-0003-0989-0620
How to cite this article:
Abed ES, Ali MR. Molecular Analysis of Whole Genomic Sequencing for *Acinetobacter Baumannii* Isolates under Antibiotic Stress in a Hospital Outbreak. J Commun Dis. 2024;56(1):106-119.

Date of Submission: 2023-09-26
Date of Acceptance: 2023-10-02

A B S T R A C T

Introduction: A study was conducted to identify specialised genes in two types of *Acinetobacter baumannii*, namely MDR, XDR, and PDR. These genes may play a crucial role in the ability of these species to coexist with the human host and show a wide range of diversity in genes that contribute to antibiotic resistance and lipopolysaccharide barrier.

Methodology: The identification of 70 *A. baumannii* isolates was carried out through morphology and culture on CHROM agar, followed by biochemical testing. Genotypic identification was performed using 16S rRNA and blaOxa-51 gene for *A. baumannii* species. Based on the antibiotic resistance categories, two isolates (AB32 and AB51) were selected for whole genome sequencing (WGS) using Illumina MiSeq technology. The analysis identified specialised genes in these isolates that contribute to antibiotic stress and lipopolysaccharide barrier, including complex sets of partial and complete integrons and transposons.

Results: The recent findings showed that *A. baumannii* exhibits the highest resistance to gatifloxacin. The genome analysis revealed that AB3, AB32, and AB51 belong to unique STs (ST/1418, and ST/441), while AB R75 belongs to a known international clone of a high-risk strain (ST/195). The presence of efflux pump genes EmrAB-TolC, MacA, MacB, MdfA/ Cmr, TolC/ OpmH, and atG catalase gene encoding an antibiotic activation enzyme was identified in the WGS results. In addition, antibiotic target protection and replacement proteins that confer resistance against gatifloxacin and colistin sulphates were encoded by BcrC, FabG, and HtdX genes.

Conclusions: Our study provided a detailed genomic picture of both innate and acquired plasmid-encoded AMR genes.

Keywords: *Acinetobacter baumannii*, Whole Genomic Sequence (WGS), Antibiotic Resistance Genes

Introduction

Acinetobacter baumannii is a gram-negative coccobacillus that is non-motile, catalase-positive, strictly aerobic, and negative for glucose fermentation, oxidase, urease, citrate, and indole. The DNA sequence of *A. baumannii* contains 39%–47% guanine-cytosine (GC).[1] In recent decades, *A. baumannii* has emerged as a highly problematic nosocomial pathogen around the world, causing a majority of nosocomial infections as a result of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug resistant (PDR) strains. Risk factors for the infection include prolonged stays in the intensive care unit, prolonged antibiotic exposure, mechanical ventilation, use of a central venous catheter, haemodialysis, and in most cases, colonisation in respiratory secretions of ventilated patients. *A. baumannii* is a highly virulent pathogen and is capable of causing infection in immunocompromised and neutropenic patients.[2,3]

The standard genome size of *A. baumannii* ranges from 3.7 to 4.3 Mb, with a median GC content of 39%.[4] However, due to its extreme genome plasticity and the ability to acquire foreign DNA, the genome size may be enlarged to reach 9 Mb or more. *A. baumannii* has a small core genome and a large accessory genome.[5]

Molecular epidemiology provides an essential tool for the elimination or control of *A. baumannii* strains inside medical centres. Various molecular typing techniques, such as whole-genome sequencing (WGS), pulsed-field gel electrophoresis (PFGE), and multi-locus sequence typing (MLST), have been used to epidemiologically characterise microorganisms responsible for hospital-acquired infections (HAIs), including strains of A. baumannii. The use of Illumina platforms, which are classified as second-generation sequencers, has made important contributions to the field of molecular epidemiology in the study of *A. baumannii* strains. Whole genome sequencing (WGS) gives an advanced and detailed approach to studying several aspects of the *A. baumannii* pathogen. It enables the identification of the pathogen, analysis of its virulence factors, determination of medication susceptibility, and examination of comparative genomics, and facilitates the detection and investigation of outbreaks.[6,7]

To understand the resistance mechanism for *A. baumannii* species, this study aims to establish the genetic makeup of the species. Various molecular typing methods, including WGS, PFGE, and MLST, have been utilised to investigate the epidemiology and resistance genes of *A. baumannii* strains.[8,9]

Methodology

Bacterial Isolates

Between February and April 2022, a total of 70 non-repetitive A. baumannii isolates were obtained from 150 clinical specimens such as CSF, blood, sputum, and urine from patients (ages ranging from 25 to 60 years) hospitalised in ICUs of three medical centres (The City of Medicine, Baghdad Hospital, and Al-Hurok Hospital) in different regions of Iraq, Baghdad. The isolates were subjected to characterisation using the VITEK 2 system. This included analysing the biochemical reactions of the bacterial isolates that were suspended in the appropriate solutions and medium included on VITEK 2 identification cards. The specimens were transferred to the laboratory using a transport medium and then cultivated on *A. baumannii* selective media (Chrom agar and MacConkey agar). The cultures were then incubated at a temperature of 37 °C for a duration of 24 hours while maintaining aerobic conditions. Subsequently, an analysis was conducted on the colour, form, edges, and texture of the colony.[10]

Standard Disc Diffusion Technique

An antibiotic susceptibility test was conducted on *A. baumannii* isolates through a disc diffusion test using 15 antibiotics in different concentrations. All antibiotics can be classified into the following classes: carbapenems (meropenem (MEM) 10 mg and imipenem (IMP) 10 mg), fluoroquinolones (ciprofloxacin (CIP) 10 mg and levofloxacin (LEV) 5 mg), aminoglycosides (gentamicin (CN) 10 mg), and beta-lactams (cefotaxime (CTX) 30 mg, tigecycline (TGC) 15 mg, polymyxin (PB) 100 mg, colistin sulphate (CS) 10 mg, amoxicillin/ clavulanic acid (AMC) 20/ 10 mg, ceftriaxone (CRO) 30 mg, doripenem (DOR) 10 mg, amikacin AK 30 mg, minocycline (TE) 30 mg and gatifloxacine (GET) 5 mg). The results were interpreted using the Clinical and Laboratory Standards Institute (CLSI) categories.[11]

DNA Extraction and Sequencing

There were two *A. baumannii* isolates (32 and 51) available for WGS. For DNA extraction, the High-Pure template preparation kit (Roche Applied Sciences, Mannheim, Germany) was used according to the manufacturer's instructions. To assess the quality of samples for use in later processes, the quantitation of extracted DNA was detected using a nanodrop spectrophotometer. The two *A. baumannii* strains underwent library preparation and paired-end sequencing on an Illumina MiSeq sequencer.[12] Briefly, the sequencing library was created using the Illumina TruSeq Nano DNA kit (Illumina, Inc., San Diego, CA, USA), and then paired-end sequencing was performed on an Illumina MiSeq sequencer (Illumina, San Diego, CA, USA). The search items that followed the Library Layout (paired), Library genomic Source, and Illumina were added to the results.

Bioinformatics Data Analysis

The pipeline WGSBAC (v2.0.0) was used to analyse the data from two isolates of *A. baumannii* that were sequenced for this investigation.[12] In essence, WGSBAC used FastQC (v0.11.5) to control the quality of the raw sequencing

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202415

data, and the coverage was determined.[13] Shovel (v1.0.4) was constructed using SPAdes (v3.14.0).[14] QUAST (v5.0.2) was used to inspect the quality of the assembly.[15] The databases MiniKraken (v2) and Kraken 2 (v2.0.7 beta) were used to categorise readings and assemblies and check for contamination.[16,17] 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, annotated the assembled genome sequence.[18]

The virulence characteristics were discovered using the VFDB database.[19] The GC-Profile tool was used to identify the genomic sequence's areas with aberrant G + C contents.[20,21] PHASTER[22] and two web tools were used to find prophage areas and CRISPR-Cas proteins, respectively. To check for the existence of plasmids and plasmid replicons, PlasmidFinder and Platon were employed.[23–25]

Results and Discussion

Isolation and Identification

A total of 85 clinical isolates were primarily identified as gram-negative, non-fermenting Acinetobacter spp. Out of the total samples, 70 (82.34%) infections were found to be due to *Acinetobacter baumannii* isolated from three hospitals in Baghdad. The primary diagnoses were distributed according to sources of isolation: sputum (27%), blood (25%), urine (23%) and CSF (10%). The initial diagnosis of the isolates was formulated using a combination of bacteriological and biochemical tests. Based on these tests, it was ascertained that there was a difference between the results of isolation from the hospitals. The chemical and agricultural tests have been synchronised with the genetic diagnoses which confirms that they provide an accurate final detection as compared to laboratory culture and biochemical tests on a sample obtained from a patient.

The identification and characterisation of the isolates were carried out according to certain morphology, and cultural and biochemical tests. To ensure this diagnosis of A. baumannii, isolates were preliminarily cultured on CHROM agar, Blood agar, MacConkey agar, Kligler iron agar and Cetrimide agar plates in aerobic conditions at 37 °C for 24 hours. The genetic identification of *A. baumannii* for all isolates was done by housekeeping gene 16S rRNA to detect the genus of Acinetobacter and by blaOxa51 to detect the species of *Acinetobacter baumannii* using the PCR technique. PCR assays have been used in fingerprinting with 100% specificity and sensitivity for their intended targets.

Antibiotic Sensitivity Tests

Standard Disk Diffusion

Disc diffusion test was used to conduct antibiotic susceptibility testing on all 70 clinical isolates of A. baumannii. The study identified a high level of resistance in *A. baumannii* clinical isolates to most of the antibiotics tested. The results presented in Figure 1 showed that the highest resistance of *A. baumannii* was to gatifloxacin (GET) (69, 98.57%), followed by cefotaxime (CTX) (68, 97.13%), amoxicillin/ clavulanic acid (AMC) (68, 97.13%), ceftriaxone (CRO) (68, 97.13%), amikacin (AK) (67, 95.71%), doripenem (DRO) (67, 95.71%), minocycline (TE) (95.00%), meropenem (MEM) (66, 94.27%), and colistin sulphate (CS) (66, 94.27%). These values were higher than those reported in other studies, which observed resistance rates of 79% for ceftriaxone, 74.3% for cefotaxime, 69.5% for minocycline, 61.9% for amoxicillin/ clavulanic acid, 60% for doripenem, and 55.2% for ceftazidime.[26] The study also found a moderate resistance to imipenem (IMP) (61, 87.14%), gentamicin (CN) (59, 84.28%), levofloxacin (LEV) (56, 80%), and polymyxin (PB) (52, 74.3%).

A few *A. baumannii* isolates were found to be sensitive to ciprofloxacin (CIP) (25, 35.71%) and tigecycline (TGC) (26, 37.21%). However, another study reported that 96.2% of the isolates were resistant to ciprofloxacin and 98.4% to tigecycline. In conclusion, ciprofloxacin and tigecycline were found to be the most effective antibiotics against A. baumannii. Whole-genome sequencing (WGS) technology was used to investigate the genes associated with antimicrobial resistance.

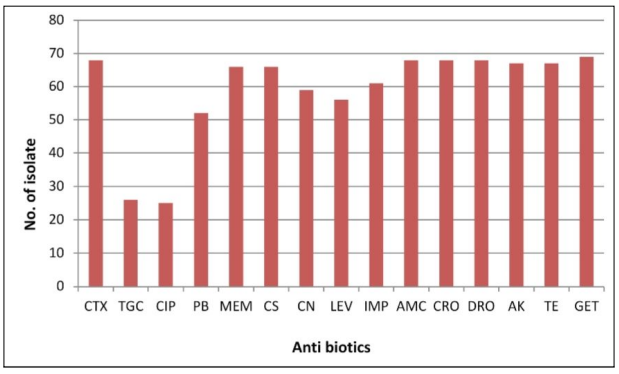

Figure 1.Antibiotic Resistance of Acinetobacter baumannii Isolates

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202415

Selected Isolates for Whole Genome Sequencing

From the results of the antibiotic resistance categories, we selected two isolates A.baumannii 32 and 51 based on their diverse outcomes within each chosen category, and these have been presented in Table 1.

Table 1.Criteria Used to Select Isolates for WGS

Character of A. baumannii isolates	A. baumannii 32	A. baumannii 51
Source isolates	From blood	From urine
Gender	Male	Female
Resistance categories	Pan drug resistance	Extensive drug resistance

Multi-locus Sequence Typing

In this study, we used the Pasteur MLST scheme to assign sequence types (STs) to the genomes of *A. baumannii* isolates. The STs were extracted from the assembled contigs for all isolates, concatenated, and aligned using the maximum-likelihood method in MEGA. Additionally, we performed core genome MLST (cgMLST) using the cgMLST scheme in the Ridom SeqSphere+ v8.0.2 software.[27] We also determined sequence types for all *A. baumannii* isolates using the database available on the Institut Pasteur MLST website.

Figure 2(a) shows 18 globally registered clones having the same sequence type (ST/1418) distributed mostly in Turkey, Ghana, and Thailand, and two novel clones with a different sequence type (ST/234) but genetically close to it, located in the same place and distributed in Sudan. The possibility of infection being transmitted by travellers or working hands to Iraq cannot be ruled out.

Figure 2(b) shows that two globally registered clones have the same sequence type (ST/441) distributed mostly in America and Germany, possibly transmitted to Iraq by American soldiers or their genetic *A. baumannii* alliance.

Both *A. baumannii* isolates in the study were subjected to MLST using seven housekeeping genes: gltA, gyrB, gdhB, recA, cpn60, gpi, and rpoD. Two globally distributed types, IS2/1418 and IS3/441, were detected in Iraqi hospitals and variations were detected through single nucleotide polymorphism (SNP) analysis compared to the typical ABR79 clonal complex (ST/195). Table 2 shows the allele number changes. IS3/441 was found to be the closest to R79. A previous study also suggested that the gpi gene was not a good candidate for MLST analysis due to recombination. Therefore, the diversity observed in *A. baumannii* suggests that the current MLST scheme may need to be further optimised, particularly with regard to the gpi gene, which may not be an ideal target for Acinetobacter MLST.

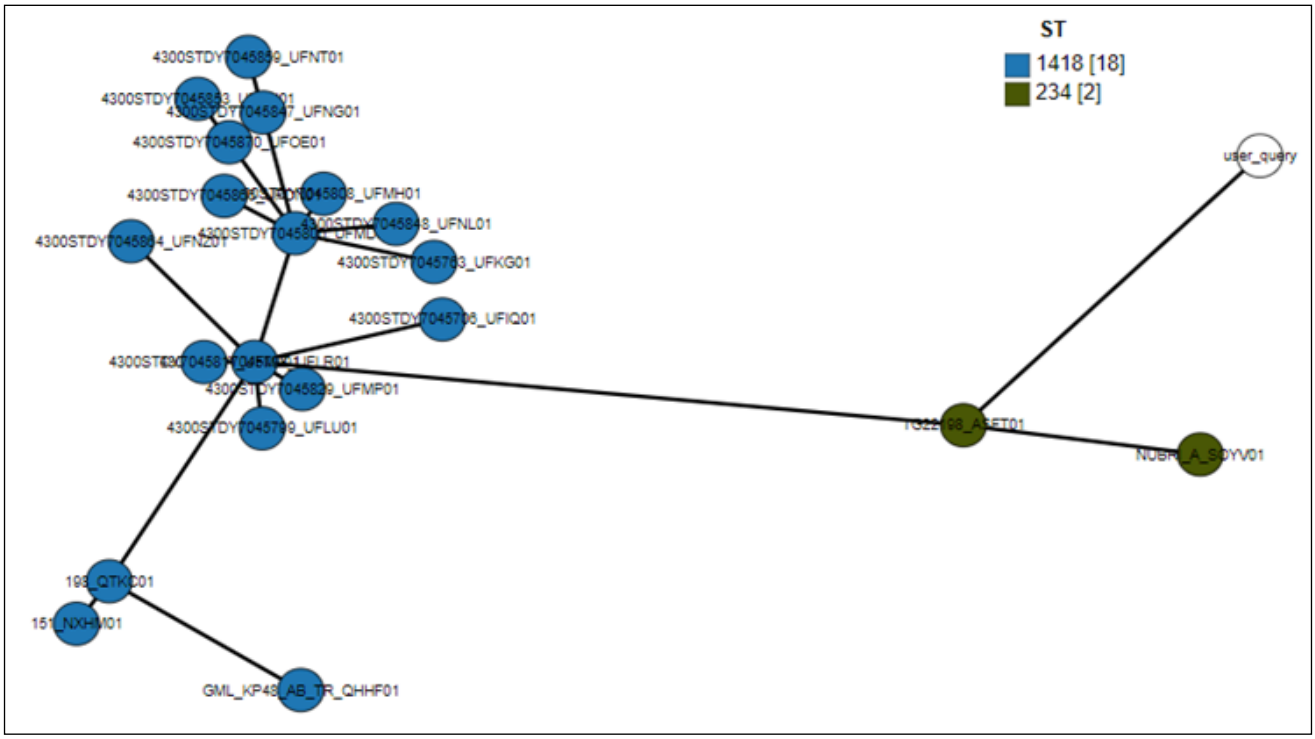

Figure 2(a).Minimal Spanning Tree for the A. baumannii 32 Isolate Based on cgMLST Characteristics. The Numbers Reflect the Amount of Distinct Alleles Found in the Pairs of Related Isolates. Close Isolates are Shown in the Same Colour; Other Isolates are Not

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202415

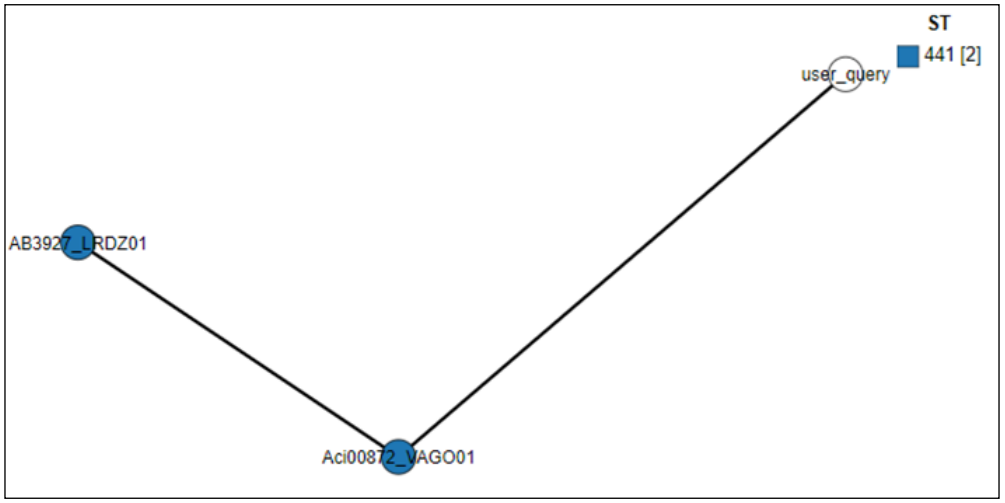

Figure 2(b).Minimal Spanning Tree for the A. baumannii 51 Isolate Based on cgMLST Characteristics. The Numbers Reflect the Amount of Distinct Alleles Found in the Pairs of Related Isolates. Close Isolates are Shown in the Same Colour; Other Isolates are Not

Information of Closely Related Isolates Based on cgMLST Strategy

Information of Isolates Closely Related to IS1-AB32 Isolate

Table 2a shows globally registered clones that have the same sequence type as our isolates AB32 (ST-1418) distributed mostly in Thailand, Turkey, and Ghana collected between 2015 and 2017, along with two novel clones distributed in Sudan with a different sequence type (ST-234) but genetically close to it, that were isolated from blood and sputum, and caused pneumonia. The possibility of infection being transmitted by travellers or working hands to Iraq cannot be ruled out.

Table 2a.Prediction of Isolates Closely Related to AB32

Isolate	Accession number	Sequence Type	Host	Disease	Isolation Source	Country State	Collection Year	Antimicrobial Resistance Gene	Virulence Gene	Different Alleles
4300STDY7045808	UFMH01	1418	Human	-	-	Thailand	2016	Show/ hide	Show/ hide	171
151	NXHM01	1418	Human	UTI	Urine	Ghana: Kumasi	2015	Show/ hide	Show/ hide	173
4300STDY7045847	UFNG01	1418	Human	-	-	Thailand	2016	Show/ hide	Show/ hide	173

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202415

Isolate	Accession number	Sequence Type	Host	Disease	Isolation Source	Country State	Collection Year	Antimicrobial Resistance Gene	Virulence Gene	Different Alleles
4300STDY7045859	UFNT01	1418	Human	-	-	Thailand	2016	Show/ hide	Show/ hide	173
4300STDY7045848	UFNL01	1418	Human	-	-	Thailand	2016	Show/ hide	Show/ hide	174
4300STDY7045870	UFOE01	1418	Human	-	-	Thailand	2016	Show/ hide	Show/ hide	175
GML_KP48_AB_TR	QHHF01	1418	Human	-	Wound infection	Turkey: Kayseri	2017	Show/ hide	Show/ hide	175
TG22198	ASFT0	234	Human	-	Blood	Sudan: Khartoum	-	Show/ hide	Show/ hide	72

Information of Isolates Closely Related to IS3-AB51

As seen in Table 2b, there are two globally registered clones that have the same sequence type (ST-441) as our isolate AB51, isolated from different clinical material, distributed mostly in America and Germany, and possibly transmitted to Iraq by American soldiers or their genetic AB alliance.

Whole Genome Sequencing of three *A. baumannii* Strains

Whole genome sequencing (WGS) is a powerful method for identifying genetic components of nosocomial pathogens and is increasingly used in clinical microbiology laboratories. In this study, *A. baumannii* isolates were sequenced using Illumina MiSeq technology with 2 × 250 bp reads and 300 bp insert size. Using the NCBI Prokaryotic Genome Automatic Annotation Pipeline (PGAAP) and the genome of *A. baumannii* strain GU71 (470.4681) as a reference sequence, 9,173, and 3,982 protein-coding sequences were predicted for *A. baumannii* 32, and *A. baumannii* 51, respectively.[28] The genomes of 32 had complete ribosomal clusters with 43 tRNAs, while *A. baumannii* 51 had 62 tRNAs. Additionally, *A. baumannii* 32 had 9 tRNAs, while *A. baumannii* 51 had 2 rRNAs.

The resulting genomes of *A. baumannii* 32 and *A. baumannii* 51 have been shown in Table 3. The quality of the assembled genomes was good. G + C content is an important criterion in WGS, and any changes in it can indicate mutation, recombination, or acquisition of mobile genetic elements from related species. The high G + C content in 32 genomes is likely due to the acquisition of additional genes from other bacterial isolates through horizontal gene transfer (integrons and transposons) during their several-year stay in the hospital, while *A. baumannii* 51 has conserved G + C content within the standard range, indicating a wild-type genome that has not undergone mutation or horizontal gene transfer.

Table 2b.Prediction of Isolates Closely Related to AB51

Isolate	Accession number	Sequence Type	Host	Disease	Isolation Source	Country State	Collection Year	Antimicrobial Resistance Gene	Virulence Gene	Different Alleles
Aci00872	VAGO01	441	Human	-	Clinical material	Germany: Heidelberg	2015-09-12	Show/ hide	Show/ hide	347
AB3927	LRDZ01	441	Human	Infection	STS	USA: Washington DC	2007-05-18	Show/ hide	Show/ hide	473

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202415

Table 3.Genome Features of A. baumannii 32 & 51 Strains

Assembly Details	A. baumannii 32	A. baumannii 51
Contigs	86	153
GC content	49.80	38.91
Contig L50	8	18
Genome length (bp)	9,269,118	4,066,533
Contig N50	348,792	75,929
Genome quality	Good	Good
CDS	9,173	3,982
tRNA	143	62
Repeated region	41	28
rRNA	9	2

Prevalence of Antimicrobial Resistance Genes

The PATRIC Genome Annotation Service detects AMR genes using a k-mer-based method that uses a curated collection of AMR gene sequence variants. This service assigns each AMR gene with functional annotations, broad mechanism of antibiotic resistance, drug class and, in some cases, specific antibiotics to which it confers resistance. It is important to consider specific AMR mechanisms and the presence or absence of SNP mutations that convey resistance. Table 4 provides a summary of the AMR genes and their corresponding mechanisms annotated in the genomes of *A. baumannii* 32 and 51. Based on the information provided in the table, it is difficult to determine which isolate is more antibiotic-resistant without additional context. Both *A. baumannii* 32 and *A. baumannii* 51 have a high number of genes associated with antibiotic resistance but the specific genes and mechanisms of resistance differ between the isolates.

The catalase gene KatG, responsible for antibiotic activation, was found in two *A. baumannii* 32 and 51 isolates. These cells induced a more robust respiratory burst in neutrophils and were more virulent than the wild-type *A. baumannii* strain. The BcrC gene, associated with peptidoglycan biosynthesis, was also found in these isolates and played an important role in biofilm formation for the protection of the antibiotic-target peptidoglycan cell wall. Additionally, the FabG and HtdX proteins were found to be important for *A. baumannii* survival in the presence of antibiotic stressors and limited nutrient conditions.

The giBd gene, considered to confer low-level streptomycin resistance, was found in two isolates but confers resistance via absence. A point mutation in the giBd gene encoding S-adenosyl methionine (SAM)-dependent 7-methylguanosine (m7G) methyltransferase, required for the methylation of 16S rRNA, confers streptomycin resistance.[29] The GdpD and PgsA proteins were found to be important for altering cell wall charge conferring antibiotic resistance in both genomes.[30] The OxyR gene was defined as a transcriptional regulator of the H_2O_2 stress response and is a direct regulator of the KatG gene, which encodes the major H_2O_2-degrading enzymes in A. baumannii. An OxyR mutant was less fit than wild-type *A. baumannii* during infection of the murine lung. The gyrB was validated for easy identification of *A. baumannii* in comparison with gold standard WGS-based assays.[31] The outer membrane immunogenic proteins OprQ, OprB, and OprD family modulate the permeability to antibiotics and are major proteins conferring bacteria host-fitness advantages including immune evasion, stress tolerance, and resistance to antibiotics and antibacterials. EmrAB-TolC, MacA, MacB, MdfA/ Cmr, TolC/ OpmH 5 are efflux pump systems that contribute to antibiotic resistance in A. baumannii. The deletion of the MacB transporter was, however, shown to be associated with a slight decrease in erythromycin resistance, for example, *A. baumannii* 32 has genes associated with resistance to ADC, CTX-M, Mph(A), OXA-51, and SHV families of antibiotic inactivation enzymes, while *A. baumannii* 51 has genes associated with resistance to ADC, OXA-23, and OXA-51 families of antibiotic inactivation enzymes.[32] Similarly, the isolates differ in the number and type of efflux pumps and proteins modulating permeability to antibiotics. *A. baumannii* 32 has the most efflux pumps conferring antibiotic resistance (9) and also has the most protein-modulating permeability to antibiotics (5). Therefore, it would be necessary to evaluate the specific antibiotic resistance profiles of each isolate in a clinical setting to determine which one is more antibiotic-resistant.

Table 4.Antimicrobial Resistance Genes

AMR Mechanism	*baumannii 32 Genes*	*baumannii 51 Genes*
Antibiotic activation enzyme	*KatG*	*KatG*
Antibiotic inactivation enzyme	*ADC family, CTX-M family, Mph(A) family, OXA-51 family, SHV family*	*ADC family, OXA-23 family, OXA-51 family*

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202415

Antibiotic target in susceptible species	*Alr, Ddl, Dxr, EF-G, EF-Tu, Fola, Dfr, Folp, gyrA, gyrB, Inha, Fabi, Iso-tRNA, Kasa, MurA, rho, rpoB, rpoC, S10p, S12p*	*Alr, Ddl, Dxr, EF-G, EF-Tu, Fola, Dfr, Folp, gyrA, gyrB, Inha, Fabi, Iso-tRNA, MurA, rho, rpoB, rpoC, S10p, S12p*
Antibiotic target protection protein	*BcrC*	*BcrC*
Antibiotic target replacement protein	*FabG, HtdX*	*FabG, HtdX*
Efflux pump conferring antibiotic resistance	*AcrAB-TolC, AcrAD-TolC, AcrEF-TolC, AcrZ, EmrAB-TolC, EmrD, MacA, MacB, MdfA/ Cmr, MdtABC-TolC, MdtL, MdtM, QacE, SugE, TolC/ OpmH 13*	*EmrAB-TolC, MacA, MacB, MdfA/ Cmr, TolC/ OpmH 5*
Gene conferring resistance via absence	*GidB*	*GidB*
Protein-altering cell wall charge conferring antibiotic resistance	*GdpD, PgsA*	*GdpD, PgsA*
Protein modulating permeability to antibiotic	*OccD6/ OprQ, OprB, OprD family*	*OprB, OprD family*
Regulator modulating expression of antibiotic resistance genes	*AcrAB-TolC, EmrAB-TolC, H-NS, OxyR*	*OxyR*

Table 5a.Best Characterised Regulatory Systems in Acinetobacter baumannii 32 (IS1)

Gene Name	Result	Type	Function
P1 dnaA	Chromosomal replication initiator protein dnaA	DNA-binding protein	dnaA bound at oriC forms a homo-oligomer that mediates an open complex formation and allows the assembly of an initiation complex that loads the replicative helicase.[31]
dnaN, recF	DNA polymerase III sub-unit beta, DNA replication/ repair protein recF	DNA-binding protein	The gene encoding β, dnaN, maps between dnaA and recF, which are involved in the initiation of DNA replication at oriC and resumption of DNA replication at disrupted replication forks, respectively.[32]
gyrB	DNA topoisomerase (ATP-hydrolysing) sub-unit B	DNA-binding protein	gyrB genes catalyse the interconversion of DNA topoisomers through relaxation, supercoiling, catenation, and decatenation. Thereby, they play a crucial role in maintaining the structural integrity of the DNA during processes that require strand separation, such as DNA replication and transcription.[33]
glyQ	glycine-tRNA alpha sub-unit	RNA-binding protein	Aminoacyle-tRNA biosynthesis[34]
gmhB	D-glycero-beta-D-manno-heptose 1,7-bisphosphate 7-phosphatase	Transcriptional Factor	gmhB supports two divergent biochemical pathways in bacteria: the d-glycero-d-manno-heptose-1α-GDP pathway (in S-layer glycoprotein biosynthesis) and the l-glycero-d-manno-heptose-1β-ADP pathway (in lipid A biosynthesis).[35]

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202415

sctL	Type III secretion system stator protein sctL	Transcriptional Factor	SctL from the flagellum, which is a key organelle for bacterial motility, and the core components that are involved in the assembly of these complex nanomachines are highly conserved.[36]
sctI	Type III secretion system inner rod sub-unit sctI	Transcriptional Factor	Needle-filament formation and length regulation[37]
sctR	Type III secretion system export apparatus sub-unit sctR	Transcriptional Factor	The export apparatus is composed of five distinct proteins: sctR, sctS, sctT, sctU and sctV, collectively facilitating a proposed structural framework for substrate gating.[38]
sctS	Type III secretion system export apparatus sub-unit sctS	Transcriptional Factor	
sctT	Type III secretion system export apparatus sub-unit sctT	Transcriptional Factor	
trkA	Trk system potassium transporter trkA	Transcriptional Factor	The adjacent trkA and trxB genes each encode a pyridine nucleotide-disulphide reductase (Pfam PF13738 and PF07992, respectively), whose precise function is currently unknown, and may also be involved in the removal of arsenic oxyanions.[39]
fmt	Methionyl-tRNA formyltransferase	Nucleoid-associated protein	Attaches a formyl group to the free amino group of methionyl-tRNA(fMet). The formyl group appears to play a dual role in the initiator identity of N-formylmethionyl-tRNA by promoting its recognition by IF2 and preventing the misappropriation of this tRNA by the elongation apparatus.[40]
rsmB	16S rRNA (cytosine (967)-C(5))-methyltransferase rsmB	Nucleoid-associated protein	A small non-coding regulatory RNA molecule, rsmB (rsmZ), works with rsmA to regulate the expression of several virulence-related genes, including the N-acyl-homoserine lactone synthase genes lasI and rhlI, and the hydrogen cyanide and rhamnolipid biosynthetic operons. rsmB is the small, untranslated regulatory RNA molecule that works in tandem with rsmA. rsmB is proposed to bind and sequester rsmA, and hence limit its activity. Therefore, gacA also regulates the activity of the rsmA protein by controlling the levels of its partner molecule, rsmB.[41]
dprA	DNA-processing protein dprA	DNA-binding protein	The product of the gene is a hypothetical protein. This gene works with comEA, comA, comF, priA, dprA, and recA genes in DNA uptake and processing.[42]

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202415

hemF	Oxygen-dependent coproporphyrinogen oxidase	Transcriptional Factor	It works with HemNand HemZ in the synthesis of protoporphyrinogen IX from coproporphyrinogen III.[43]
aroE	Shikimate dehydrogenase	Transcriptional Factor	A house-keeping gene encoding the shikimate dehydrogenase[44]

Regulation Systems and Mobile Genetic Elements (MGE)

Controlling of F-plasmid Genes Transfer in *A. baumannii*

HGT MGE in general and plasmids in particular can explain how *A. baumannii* can colonise and adapt to various environmental conditions. The plasmids are responsible for the propagation of different traits, such as antibiotic resistance, specific degradation pathways, symbiosis and virulence, by regulating the transfer and exchange of genetic material within microbial populations. In order to better understand the features of gene flux in ecosystems, studies concentrating on plasmid maintenance, diffusion, acquisition, and loss, we must explain the controlling results of regulatory systems.

A. baumannii can undergo conjugation with the help of plasmid genes with the same species, transformation with related species (after the release of DNA by lysis of its host) or transduction (by the packaging of plasmid DNA into phage particles). The regulatory systems express their transfer genes in response to specific stimuli. For the pheromone-responsive F-plasmids of AB, it is the small peptide signals from potential recipients which trigger the conjugative transfer genes.

Finally, the transfer of plasmid genes is induced by the regulatory system to which the plasmid elements confer adaptation, for example, F-plasmid transfer in *A. baumannii* is induced by antibiotic stress under the control of a transcriptional factor regulation system in order to develop antimicrobial drug resistance. Understanding these control circuits may help to modify the management of microbial communities where plasmid transfer is either desirable or undesirable.

Regulation System Types

In this study, we identified a dozen plasmid genes in the AB isolates IS1-AB32 and IS2-AB51, by analysing the F-plasmids sequence in NCBI BLASTN and detecting genes in the gene bank. These genes could provide insight into plasmid-mediated antibiotic resistance and adaptation of *A. baumannii* to changing environments.

The selected *A. baumannii* isolates, IS1-AB32 and IS2-AB51, contain five global regulator systems that could serve as potential therapeutic targets due to their roles in mediating AB adaptation to changing environments, controlling virulence and specificity to AB bacteria (Tables 5(a) and 5(b)). These systems include two-component systems (TCS), transcriptional factor TF, RNA polymerase binding protein, nucleoid-associated protein (NAP), and DNA polymerase binding protein.

Table 5b.Best Characterised Regulatory Systems in Acinetobacter baumannii 51 (IS2)

Gene Name	Result	Type	Function
aadB	Aminoglycoside nucleotidyltransferase	Two-component system	aadB genes show resistance to both tobramycin and gentamicin.[45]
hupA	DNA-binding protein HU-alpha	DNA-binding protein	Change in growth temperature, in addition to iron availability, is an environmental cue controlling the expression of the hupA gene.[46]
dadA	D-amino acid dehydrogenase	Transcriptional regulator factor	D-Amino acid dehydrogenase is a flavoenzyme that digests free neutral D-amino acids yielding corresponding 2-oxo acids and hydrogen.[47]
lysA	Diaminopimelate decarboxylase	Transcriptional factor	Encodes diaminopimelate decarboxylase[48]
argH	Argininosuccinate lyase	Transcriptional factor	It is involved in the biosynthesis of arginine and can also render cells hypersusceptible to beta-lactam antibiotics.[49]

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202415

Subsystem Analysis

Figure 3 displays the distribution of the genome annotations. This includes, from outer to inner rings, the contigs, CDS on the forward strand, CDS on the reverse strand, RNA genes, CDS with homology to known antimicrobial resistance genes, CDS with homology to known virulence factors, GC content and GC skew. The colours of the CDS on the forward and reverse strands indicate the subsystem that these genes belong to as seen in subsystems shown ahead. A subsystem is a set of proteins that together implement a specific biological process or structural complex and PATRIC annotation includes an analysis of the subsystems unique to each genome. An overview of the subsystems for this genome is provided in Figures 3(a) and 3(b). There are several housekeeping and acquired genes related to bacterial defence mechanisms that were also observed in A. baumannii. Metabolism-associated genes were found in AB isolates 32 and 51. *A. baumannii* 32 has a high percentage of metabolic genes (1969) compared to AB 51 (761). Similar is the case with genes related to protein processing, stress response, defence, virulence, energy, membrane transporter, DNA and RNA processing, cellular processes, miscellaneous, cell envelope and regulation cell signalling.

In this article, we used a metagenomic subsystem analysis to obtain functional gene data and subsequently explain the difference between pathogenic and less pathogenic strains, and described the *A. baumannii* diversity and antimicrobial resistance over a long period in Iraqi hospitals. The protein processing genes were 486 in AB 32 and 20 in AB 51. Each subsystem contains a set of proteins that realise a specific biological process or a structural complex.[49] Several studies have highlighted the key role of the Pho regulon not only in phosphate management but also in virulence and stress responses in many bacteria. The genes encoding energy were 643 in AB 32 and 235 in 51. The majority of membrane transporter genes were 396 in AB 32 but in AB 51, they were 173. The genes responsible for DNA processing were 210 in AB 32 and 65 in AB 51, while the genes responsible for RNA processing were 164 in AB 32 and 76 in AB 51. Cellular processer genes in AB 32 were 197 and those in AB 51 were 73. Genes associated with cell envelope were 130 in AB 32 and 33 in AB 51. Finally, regulation and cell signalling genes were 52 in AB 32 and 12 in AB 51.

The genetic differences of subsystems between Acinetobacter species in key virulence attributes may help explain why some species have a greater clinical impact.

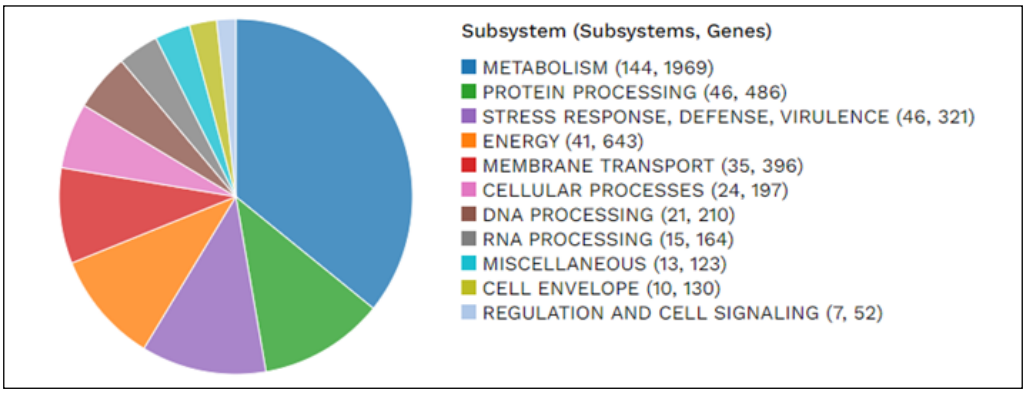

Figure 3(a).Functional Distribution Subsystem of Genes in All Three A. baumannii 32 Strains Included in This Study

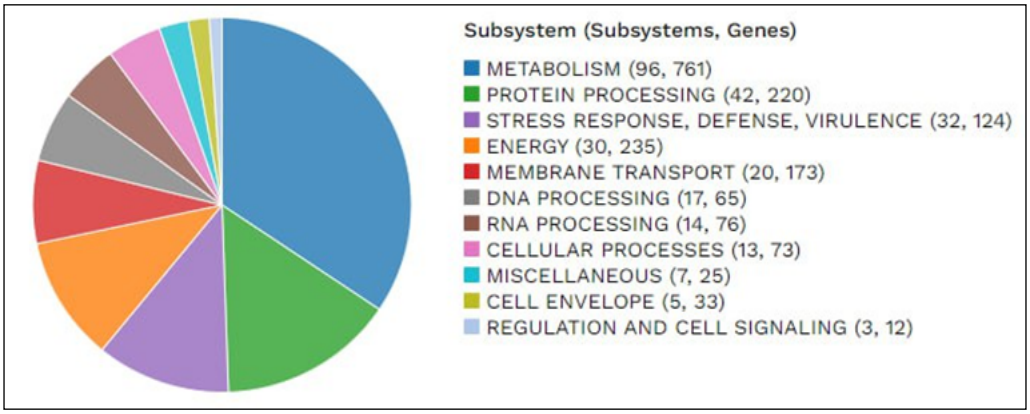

Figure 3(b).Functional Distribution Subsystem of Genes in All Three A. baumannii 51 Strains Included in This Study

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202415

Conclusion

Our study investigated the molecular epidemiology of two *A. baumannii* strains isolated from Iraqi hospitals during an outbreak of antibiotic-resistant infections, using whole-genome sequencing (WGS) analysis. The detection of both innate and acquired antimicrobial resistance (AMR) genes in these strains highlights the increased danger they pose as pathogens. Our analysis provided a detailed genomic picture of both the chromosomal and plasmid-encoded AMR genes, including those carried on mobile genetic elements.

Acknowledgements

The authors appreciate the support of the Biology Department, College of Science, Mustansiryiah University, Baghdad, Iraq, for their contribution to this research work.

Source of Funding: None

Conflict of Interest: None

References

1. Kurcik-Trajkovska B. Acinetobacter spp.-a serious enemy threatening hospitals worldwide. Maced J Med Sci. 2009;2(2):157-62. [Google Scholar]
2. Garnacho-Montero J, Timsit JF. Managing Acinetobacter baumannii infections. Curr Opin Infect Dis. 2019;32(1):69-76. [PubMed] [Google Scholar]
3. Bardbari AM, Mohajeri P, Arabestani MR, Karami M, Keramat F, Asadollahi S, Khodavirdipour A, Alikhani MY. Molecular typing of multi-drug resistant Acinetobacter baumannii isolates from clinical and environmental specimens in three Iranian hospitals by pulsed field gel electrophoresis. BMC Microbiol. 2020;20(1):101. [PubMed] [Google Scholar]
4. Adams MD, Goglin K, Molyneaux N, Hujer KM, Lavender H, Jamison JJ, MacDonald IJ, Martin KM, Russo T, Campagnari AA, Hujer AM, Bonomo RA, Gill SR. Comparative genome sequence analysis of multidrug-resistant Acinetobacter baumannii. J Bacteriol. 2008;190(24):8053-64. [PubMed] [Google Scholar]
5. Imperi F, Antunes LC, Blom J, Villa L, Iacono M, Visca P, Carattoli A. The genomics of Acinetobacter baumannii: insights into genome plasticity, antimicrobial resistance and pathogenicity. IUBMB Life. 2011;63(12):1068-74. [PubMed] [Google Scholar]
6. Bartual SG, Seifert H, Hippler C, Luzon MA, Wisplinghoff H, Rodriguez-Valera F. Development of a multilocus sequence typing scheme for characterization of clinical isolates of Acinetobacter baumannii. J Clin Microbiol. 2005;43(9):4382-90. [PubMed] [Google Scholar]
7. Diancourt L, Passet V, Nemec A, Dijkshoorn L, Brisse S. The population structure of Acinetobacter baumannii: expanding multiresistant clones from an ancestral susceptible genetic pool. PloS One. 2010;5(4):e10034. [PubMed] [Google Scholar]
8. Quainoo S, Coolen JP, van Hijum SA, Huynen MA, Melchers WJ, van Schaik W, Wertheim HF. Whole-genome sequencing of bacterial pathogens: the future of nosocomial outbreak analysis. Clin Microbiol Rev. 2017;30(4):1015-63. [PubMed] [Google Scholar]
9. Durand G, Javerliat F, Bes M, Veyrieras JB, Guigon G, Mugnier N, Schicklin S, Kaneko G, Santiago-Allexant E, Bouchiat C, Martins-Simoes P, Laurent F, Belkum AV, Vandenesch F, Tristan A. Routine whole-genome sequencing for outbreak investigations of Staphylococcus aureus in a national reference center. Front Microbiol. 2018;9:511. [PubMed] [Google Scholar]
10. Atlas RM, Brown AE, Parks LC. Laboratory manual of experimental microbiology. USA, St. Louis: Mosby; 1997.
11. Huang HY, Wang CF, Lu PL, Tseng SP, Wang YL, Chen TC, Chang K, Tu HP, Lin SY. Clinical impact of the revised 2019 CLSI levofloxacin breakpoints in patients with Enterobacterales bacteremia. Antimicrob Agents Chemother. 2021;65(6):e00074-21. [PubMed] [Google Scholar]
12. Shayea RH, Ali MR. Whole-genome study of carbapenem-resistant Acinetobacter baumannii virulence and resistance. Iran J Med Microbiol. 2023;17(1):90-102. [Google Scholar]
13. Ridha DJ, Ali MR, Jassim KA. Molecular analysis of multidrug-resistant Acinetobacter baumannii isolated in Baghdad hospital. Int J Res Pharm Sci. 2019;10(3):1970-87.
14. Schramm ST, Place K, Montaña S, Almuzara M, Fung S, Fernandez JS, Tuttobene MR, Golic A, Altilio M, Traglia GM, Vay C, Mussi MA, Iriarte A, Ramirez MS. Genetic and phenotypic features of a novel Acinetobacter species, strain A47, isolated from the clinical setting. Front Microbiol. 2019;10:1375. [PubMed] [Google Scholar]
15. Andersen SB, Ghoul M, Griffin AS, Petersen B, Johansen HK, Molin S. Diversity, prevalence, and longitudinal occurrence of type II toxin-antitoxin systems of Pseudomonas aeruginosa infecting cystic fibrosis lungs. Front Microbiol. 2017;8:1180. [PubMed] [Google Scholar]
16. Noguchi Y, Sakiyama Y, Kawakami H, Katayama T. The Arg fingers of key DnaA protomers are oriented inward within the replication origin oriC and stimulate DnaA subcomplexes in the initiation complex. J Biol Chem. 2015;290(33):20295-312. [PubMed] [Google Scholar]
17. Villarroya M, Perez-Roger I, Macián F, Armengod ME. Stationary phase induction of dnaN and recF, two

genes of Escherichia coli involved in DNA replication and repair. EMBO J. 1998;17(6):1829-37. [PubMed] [Google Scholar]
18. Hartmann S, Gubaev A, Klostermeier D. Binding and hydrolysis of a single ATP is sufficient for N-gate closure and DNA supercoiling by gyrase. J Mol Biol. 2017;429(23):3717-29. [PubMed] [Google Scholar]
19. Fereshteh S, Goodarzi NN, Kalhor H, Rahimi H, Barzi SM, Badmasti F. Identification of putative drug targets in highly resistant gram-negative bacteria; and drug discovery against Glycyl-tRNA synthetase as a new target. Bioinform Biol Insights. 2023;17:11779322231152980. [PubMed] [Google Scholar]
20. Wang L, Huang H, Nguyen HH, Allen KN, Mariano PS, Dunaway-Mariano D. Divergence of biochemical function in the HAD superfamily: D-glycero-D-manno-heptose-1,7-bisphosphate phosphatase (GmhB). Biochemistry. 2010;49(6):1072-81. [PubMed] [Google Scholar]
21. Deng W, Marshall NC, Rowland JL, McCoy JM, Worrall LJ, Santos AS, Strynadka NC, Finlay BB. Assembly, structure, function and regulation of type III secretion systems. Nat Rev Microbiol. 2017;15(6):323-37. [PubMed] [Google Scholar]
22. Gubaev A, Hilbert M, Klostermeier D. The DNA-gate of Bacillus subtilis gyrase is predominantly in the closed conformation during the DNA supercoiling reaction. Proc Natl Acad Sci U S A. 2009;106(32):13278-83. [PubMed] [Google Scholar]
23. Hamidian M, Hall RM. The AbaR antibiotic resistance islands found in Acinetobacter baumannii global clone 1–structure, origin and evolution. Drug Resist Updat. 2018;41:26-39. [PubMed] [Google Scholar]
24. Cai Y, Chandrangsu P, Gaballa A, Helmann JD. Lack of formylated methionyl-tRNA has pleiotropic effects on Bacillus subtilis. Microbiology (Reading). 2017;163(2):185-96. [PubMed] [Google Scholar]
25. Burrowes E, Baysse C, Adams C, O'Gara F. Influence of the regulatory protein RsmA on cellular functions in Pseudomonas aeruginosa PAO1, as revealed by transcriptome analysis. Microbiology (Reading). 2006;152(2):405-18. [PubMed] [Google Scholar]
26. Schobert M, Jahn D. Regulation of heme biosynthesis in non-phototrophic bacteria. J Mol Microbiol Biotechnol. 2002;4(3):287-94. [PubMed] [Google Scholar]
27. Soufiane B, Côté JC. Discrimination among Bacillus thuringiensis H serotypes, serovars and strains based on 16S rRNA, gyrB and aroE gene sequence analyses. Antonie Van Leeuwenhoek. 2009;95(1):33-45. [PubMed] [Google Scholar]
28. Anderson SE, Sherman EX, Weiss DS, Rather PN. Aminoglycoside heteroresistance in Acinetobacter baumannii AB5075. Msphere. 2018;3(4):e00271-18. [PubMed] [Google Scholar]
29. Oh MH, Lee SM, Lee DH, Choi SH. Regulation of the Vibrio vulnificus hupA gene by temperature alteration and cyclic AMP receptor protein and evaluation of its role in virulence. Infect Immun. 2009;77(3):1208-15. [PubMed] [Google Scholar]
30. Tanigawa M, Shinohara T, Saito M, Nishimura K, Hasegawa Y, Wakabayashi S, Ishizuka M, Nagata Y. D-Amino acid dehydrogenase from Helicobacter pylori NCTC 11637. Amino Acids. 2010;38(1):247-55. [PubMed] [Google Scholar]
31. Li C, Wang H, Zhou L, Zhang Y, Song F, Zhang J. Quantitative measurement of pH influence on SalR regulated gene expression in Acinetobacter baylyi ADP1. J Microbiol Methods. 2009;79(1):8-12. [PubMed] [Google Scholar]
32. Gomez MJ, Neyfakh AA. Genes involved in intrinsic antibiotic resistance of Acinetobacter baylyi. Antimicrob Agents Chemother. 2006;50(11):3562-7. [PubMed] [Google Scholar]
33. Ohneck EJ, Arivett BA, Fiester SE, Wood CR, Metz ML, Simeone GM, Actis LA. Mucin acts as a nutrient source and a signal for the differential expression of genes coding for cellular processes and virulence factors in Acinetobacter baumannii. PLoS One. 2018;13(1):e0190599. [PubMed] [Google Scholar]
34. Zusman T, Feldman M, Halperin E, Segal G. Characterization of the icmH and icmF genes required for Legionella pneumophila intracellular growth, genes that are present in many bacteria associated with eukaryotic cells. Infect Immun. 2004;72(6):3398-409. [PubMed] [Google Scholar]
35. Wang J, Zhou Z, He F, Ruan Z, Jiang Y, Hua X, Yu Y. The role of the type VI secretion system vgrG gene in the virulence and antimicrobial resistance of Acinetobacter baumannii ATCC 19606. PLoS One. 2018;13(2):e0192288. [PubMed] [Google Scholar]
36. Lucidi M, Visaggio D, Prencipe E, Imperi F, Rampioni G, Cincotti G, Leoni L, Visca P. New shuttle vectors for real-time gene expression analysis in multidrug-resistant Acinetobacter species: in vitro and in vivo responses to environmental stressors. Appl Environ Microbiol. 2019;85(18):e01334-19. [PubMed] [Google Scholar]
37. Bedore SR, Schmidt AL, Slarks LE, Duscent-Maitland CV, Elliott KT, Andresen S, Costa FG, Weerth RS, Tumen-Velasquez MP, Nilsen LN, Dean CE, Karls AC, Hoover TR, Neidle EL. Regulation of l- and d-Aspartate transport and metabolism in Acinetobacter baylyi ADP1. Appl Environ Microbiol. 2022;88(15):e0088322. [PubMed] [Google Scholar]
38. Gipson KS, Nickerson KP, Drenkard E, Llanos-Chea A, Dogiparthi SK, Lanter BB, Hibbler RM, Yonker LM, Hurley BP, Faherty CS. The great ESKAPE: exploring the

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202415

crossroads of bile and antibiotic resistance in bacterial pathogens. Infect Immun. 2020;88(10):e00865-19. [PubMed] [Google Scholar]

39. Matsutani M, Fukushima K, Kayama C, Arimitsu M, Hirakawa H, Toyama H, Adachi O, Yakushi T, Matsushita K. Replacement of a terminal cytochrome c oxidase by ubiquinol oxidase during the evolution of acetic acid bacteria. Biochim Biophys Acta. 2014;1837(10):1810-20. [PubMed] [Google Scholar]
40. Saiki K, Mogi T, Ogura K, Anraku Y. In vitro heme O synthesis by the cyoE gene product from Escherichia coli. J Biol Chem. 1993;268(35):26041-4. [PubMed] [Google Scholar]
41. Lomovskaya O, Nelson K, Rubio-Aparicio D, Tsivkovski R, Sun D, Dudley MN. Impact of intrinsic resistance mechanisms on potency of QPX7728, a new ultrabroad-spectrum beta-lactamase inhibitor of serine and metallo-beta-lactamases in Enterobacteriaceae, Pseudomonas aeruginosa, and Acinetobacter baumannii. Antimicrob Agents Chemother. 2020;64(6):e00552-20. [PubMed] [Google Scholar]
42. Nieto JM, Carmona M, Bolland S, Jubete Y, de la Cruz F, Juarez A. The hha gene modulates haemolysin expression in Escherichia coli. Mol Microbiol. 1991;5(5):1285-93. [PubMed] [Google Scholar]
43. Gaddy JA, Tomaras AP, Actis LA. The Acinetobacter baumannii 19606 OmpA protein plays a role in biofilm formation on abiotic surfaces and in the interaction of this pathogen with eukaryotic cells. Infect Immun. 2009;77(8):3150-60. [PubMed] [Google Scholar]
44. Hsieh YJ, Wanner BL. Global regulation by the seven-component Pi signaling system. Curr Opin Microbiol. 2010;13(2):198-203. [PubMed] [Google Scholar]
45. Aguena M, Yagil E, Spira B. Transcriptional analysis of the pst operon of Escherichia coli. Mol Genet Genomics. 2002;268(4):518-24. [PubMed] [Google Scholar]
46. Arora SK, Ritchings BW, Almira EC, Lory S, Ramphal R. The Pseudomonas aeruginosa flagellar cap protein, FliD, is responsible for mucin adhesion. Infect Immun. 1998;66(3):1000-7. [PubMed] [Google Scholar]
47. Flannagan RS, Valvano MA, Koval SF. Downregulation of the motA gene delays the escape of the obligate predator Bdellovibrio bacteriovorus 109J from bdelloplasts of bacterial prey cells. Microbiology (Reading). 2004;150(Pt 3):649-56. [PubMed] [Google Scholar]
48. Allison GE, Fremaux C, Klaenhammer TR. Expansion of bacteriocin activity and host range upon complementation of two peptides encoded within the lactacin F operon. J Bacteriol. 1994;176(8):2235-41. [PubMed] [Google Scholar]
49. Pellicer MT, Badía J, Aguilar J, Baldomà L. glc locus of Escherichia coli: characterization of genes encoding the subunits of glycolate oxidase and the glc regulator protein. J Bacteriol. 1996;178(7):2051-9. [PubMed] [Google Scholar]

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202415