JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Coronavirus Pandemic

Acinetobacter baumannii clinical isolates from outbreaks in Erbil hospitals after the COVID-19 pandemic

Hazheer M Hamad[1], Hanan T Subhi[1]

[1] Department of Biology, Faculty of science and health, Koya University, Koya KOY45, Kurdistan Region - F.R. Iraq

Abstract

Introduction: *Acinetobacter baumannii* is endemic in hospital environments, and since the coronavirus disease 2019 (COVID-19) pandemic, multidrug-resistant *A. baumannii* has become more potent. This potential evolution is driven by the undetectable numbers of gene resistances it has acquired. We evaluated the antibiotic-resistance genes in isolates from patients in Erbil hospitals.

Methodology: This is the first study to demonstrate the antimicrobial resistance epidemic in Erbil, Iraq. A total of 570 patients, including 100 COVID-19 patients were tested. Isolate identification, characterization, antibiotics susceptibility test, polymerase chain reaction (PCR) amplification of the antibiotic resistance genes in both bacterial chromosome and plasmid, 16S-23S rRNA gene intergenic spacer (ITS) sequencing using the Sanger DNA sequencing, and phylogenetic analysis were used in this study.

Results: Only 13% of *A. baumannii* isolates were from COVID-19 patients. All isolates were multi-drug resistant due because of 24 resistance genes located in both the bacterial chromosome or the plasmid. *blaTEM* gene was detected in the isolates; however, *aadB* was not detected in the isolated bacteria. New carbapenemase genes were identified by Sanger sequencing and resistance genes were acquired by plasmids.

Conclusions: The study identified metabolic differences in the isolates; although all the strains used the coumarate pathway to survive. Several resistance genes were present in the isolates' plasmids and chromosome. There were no strong biofilm producers. The role of the plasmid in *A. baumannii resistance* development was described based on the results.

Key words: A. baumannii; resistance genes; carbapenemase genes; blaTEM; plasmid.

J Infect Dev Ctries 2024; 18(9.1):S56-S66. doi:10.3855/jidc.17885

(Received 10 January 2023 – Accepted 31 July 2023)

Introduction

The emergence of multidrug-resistant bacteria *Acinetobacter baumannii* has contributed to increasing hospital-acquired infections [1]. The World Health Organization (WHO) included this bacterium in the 'global priority list of antibiotic-resistant bacteria' due to its high level of resistance to all antibiotic classes resulting in difficulties in anti-infective treatment, leading to a higher possibility of developing septic shock [2,3]. Resistance rates as high as 70% have been reported in the Middle East [4]. Carbapenem-resistant *Acinetobacter baumannii* is important clinically and epidemically due to its spread in intensive care units (ICUs) via hospital environment contamination, healthcare workers' hands, and asymptomatic colonization [5]. Cancer, immunodeficiency, and COVID 19 patients are the most affected, and the morbidity and mortality of COVID-19 patients increase significantly with *A. baumannii* coinfection [6,7]. The incidence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) secondary infections along with *A. baumannii* has been reported to vary from 1 to 1.4% in different counties [8-10]. The bacteria acquire resistance mechanisms mostly through mobile genetic elements and are therefore phylogenetically variable. The bacteria can acquire integrons, transposons, resistance islands, and insertion sequences to increase their resistance [11,12].

The mechanism of action of carbapenem-resistant *Acinetobacter baumannii* includes carbapenemase hydrolysis by OXA-type carbapenemases, as well as an alteration in porin and efflux pumps [13]. Efflux pumps play a role in biofilm formation, DNA mutation, and acquisition of antibiotics resistance in *A. baumannii*. The outer membrane protein adeABC is the common efflux pump resistance against diverse antibiotics [14]. The Ambler method classifies *A. baumannii* β-lactamases using molecular structures into four groups; A, B, C, and D; however, the most potent class is the class D β-lactamases or carbapenemases which include blaOXA-51, blaOXA-23, blaOXA-24, blaOXA-58, blaOXA-143, and blaOXA-235 [15]. Class D is encoded both on the chromosome and the plasmid genes, and has been detected in most regions of the world [13,16]. Class A is mostly used in clinical settings and encodes resistance to penicillin,

cephalosporins monobactams, and carbapenems by plasmid and other DNA mobile elements. Class C is chromosomally encoded and confers resistance to cephalosporins, carbapenems and sulbactam in all *A. baumannii* and is expressed by insertion sequences ISAba1 and ISAba125. Class B in *A. baumannii* includes four types of genes; *IMP*, *VIM*, *NDM*, and *SIMl*, that are encoded in plasmids and integrons, and hydrolyze all β-lactamases [16].

The aims of this study were phylogenetic identification of the clinical isolates using biochemical tests and 16S-23S rRNA gene intergenic spacer region (ITS); evaluation of the antibiotic susceptibility profile; and genetic detection of antibiotic resistance in *Acinetobacter* spp. isolates obtained from Arzheen Private Hospital, Al Jumhury Teaching Hospital, King Medical Laboratory, and Nanakali Hospital for Blood Diseases and Cancer. Arzheen Hospital is the major hospital in Erbil and received COVID-19 patients. In addition to identifying and sequencing *OXA*-type β-lactamases gene in this bacterium, the study aimed to determine the capacity of *A. baumannii* acquired resistance genes from different bacterial species. Finally, the capability of the isolates to form biofilm was analyzed.

Methodology

Sample collection

Samples were collected from 570 patients who were treated at the four microbiology diagnostic laboratories in Erbil city (Hawler), Iraq. Both inpatients and outpatients were included in this study. *A. baumannii* were isolated from clinical samples (blood, urine, sputum, abscess, and wounds) from October 1 to December 31, 2021. Then the samples were directly transferred onto blood agar and MacConkey agar (Himedia, Mumbai, Maharashtra, India), except for blood specimens which were directly transferred into a special blood culture bottle, followed by cultivation and incubation at 37 °C for 24 hours.

Bacterial isolate identification

The identification methods were based on conventional biochemical tests, VITEK® 2 compact system (BioMérieux, Marcy l'Etoile, France), and polymerase chain reaction (PCR) [17]. All the isolates were subjected to PCR, and targeted sequence analysis of the 16S-23S rRNA gene ITS [18] (Supplementary Tables 1 and 2).

Antimicrobial susceptibility

Antimicrobial susceptibility testing was performed on the 17 isolates by the automated minimum inhibitory concentration (MIC) detection method with microbroth dilution (VITEK®, BioMérieux, Marcy l'Etoile, France). Resistance to the antibiotics piperacillin piperacillin/tazobactam, ceftazidime, cefepime, imipenem, meropenem, gentamicin, netilmicin, tobramycin, ciprofloxacin, levofloxacin, tetracycline, tigecycline, colistin, and trimethoprim/sulfamethaxole were determined following the guidelines of the Clinical and Laboratory Standards Institute (CLSI).

Biofilm formation

Quantitative biofilm formation ability of *A. baumannii* isolates was measured as described by [19]. Polystyrene microtiter plate based on the safranin staining method was applied [20]. Briefly, nutrient broth containing 1% (w/v) glucose was inoculated with the isolate's bacteria at $OD_{600} = 0.1$, and the tubes were incubated at 37 °C for 24 hours. Then, 20 µL of each isolate broth was added to the wells followed by adding 180 µL of nutrient broth without bacteria and incubated at 37 °C. After 24 hours, the broth was discarded, and the adherent bacteria were washed twice with saline water and left to dry. Then it was stained with 0.1% safranin and incubated for 10 minutes. The stain was washed using 200 µL distilled water and allowed to dry. Finally, the absorbance of each well was measured at 490 nm using a BioTic ELISA reader (EL x 800; Profcontrol GmbH, Schönwalde-Glien, Germany). The controls included cultures without bacteria. The assay was done in triplicate for each isolate.

The optical density cut-off value (ODc) was measured and biofilm formation was detected using the formula described by [21].

Genotypic identification

DNA extraction

After overnight sub-culture on MacConkey agar at 37 °C, bacterial DNA extraction was performed by boiling several identical colonies in 100 µL of sterile distilled water for about 15-20 minutes, followed by centrifuging, and then the supernatant was used as the DNA template.

Plasmid DNA extraction

A volume of 1.5 mL of overnight cultured bacterial cells was transferred to a microcentrifuge tube. The plasmids DNA were extracted by using High-Speed Plasmid Mini Kit (Geneaid, New Taipei City, Taiwan),

and visualized on a 1% agarose gel that was run at 75 V for 60 min.

Gene amplification by PCR

All PCR reactions were performed in a total volume of 25 µL, with reagents supplied by Promega (Promega, Southampton, U.K.) according to the manufacturer's guidelines. The primers used in this study were supplied by Eurofins MWG operon (Wolverhampton, U.K.; Supplementary Table S1).

Molecular identification of isolates using 16S-23S rRNA (ITS)

PCR amplification was performed with 2 µL of DNA template in an OmniGen Thermal Cycler (Hybaid Limited, Middlesex, United Kingdom). The 1512F/6R primer sets are described in Supplementary Table S1, and the PCR conditions are summarized in Supplementary Table S2. PCR products were visualized using 1.5% agarose gel electrophoresis [18], and then the amplicons were sequenced at the Immuno Gene Center (Erbil, Iraq).

Antibiotic resistance genes

Resistance genes of the isolates were determined in both the chromosomes and plasmids by PCR amplification. The isolate's resistance gene detection was performed as follows (Supplementary Tables 1 and 2):

1. OXA-type β-lactamases genes were identified and followed by sequencing of the PCR product.
2. Class A were bla_{SHV} and bla_{TEM} genes,
3. Class B was bla_{NDM} gene,
4. Class D were bla_{OXA-23}, bla_{OXA-27}, bla_{OXA-49}, bla_{OXA-58}, bla_{OXA-96}, bla_{OXA-51}, bla_{OXA-58}, bla_{OXA-64}, bla_{OXA-69}, bla_{OXA-70}, bla_{OXA-71}, bla_{OXA-75}, and bla_{OXA-78}.

Table 1. Molecular identification of isolates by analysis and sequencing amplified ITS fragments and β- lactamase genes.

Isol.	*23S/16SrRNA*	β- lactamase gene			
		blaOXA-51-like	***blaOXA-23-like***	***blaNDM***	***blaTEM***
1					*A. baumannii* strain beta-lactam resistance HK22 [5]
2		*A. baumannii* strain Abau36 OXA-51 [2]	*A. baumannii*		
3	*A. baumannii*	*A. baumannii* strain Abau36 OXA-51 [2]	*A. baumannii*		
4	*A. baumannii*	*A. baumannii* strain Abau36 OXA-51 [2]	*A. baumannii*	*A. baumannii strain IPK-9 subclass B1 metallo-beta-lactamase NDM-42 (blaNDM)*[3]	*A. baumannii*
5		*A. baumannii* strain Abau36 OXA-51 [2]			
6	*A. baumannii*				*A. baumannii*
7	*A. baumannii*	*A. baumannii* strain Abau36 OXA-51 [2]	*A. baumannii*	*A. baumannii strain IPK-9 subclass B1 metallo-beta-lactamase NDM-42 (blaNDM)*[3]	
8	*A. baumannii*				*A. baumannii* strain beta-lactam resistance HK22 [5]
9				*A. baumannii* strain carbapenems resistance HK19[4]	*A. baumannii* strain beta-lactam resistance HK22 [5]
10	*A. baumannii*	*A. baumannii* strain Abau36 OXA-51 [2]	*A. baumannii*	*A. baumannii strain IPK-9 subclass B1 metallo-beta-lactamase NDM-42 (blaNDM)*[3]	
11*	*A. baumannii*	*A. baumannii* strain Abau36 OXA-51[2]			*A. baumannii* strain beta-lactam resistance HK22 [5]
12	*A. baumannii*				
13					
14	*P. aeruginosa*				
15					
16				*A. baumannii* strain carbapenems resistance HK19[4]	
17	*S. maltophilia* strain HK[1]	*A. baumannii*[6]		*A. baumannii strain IPK-9 subclass B1 metallo-beta-lactamase NDM-42 (blaNDM),*[3,6]	

Isol., Isolates; *A.baumannii, A. baumannii; P. aeruginosa, Pseudomonas aeruginosa; S. maltophilia, Stenotrophomonas maltophilia*. *The isolate was coinfected with *E. coli*. [1]New strain in this study; Genebank accession number: OP422244. [2] New OXA-51 family class D beta-lactamase from *A.baumannii* , submitted on 25 May 2022. French National Reference Center for antibiotics resistance, University Hospital of Besancon, France. [3] Novel gene submitted on 7 March 2022 in Japan. [4] New strain of beta-lactamase (bla_{NDM}) gene in this study; Genebank accession number: OP572243. [5] *A. baumannii* new strain of beta-lactamase (bla_{TEM}) gene in this study; Genebank accession number OP572244. [6] New strain of *S. maltophilia* beta-lactamase $bla_{oxa\ 51}$ like gene and bla_{NDM} gene in this study; Genebank accession numbers OP595162, OP595163.

Antibiotic resistance genes detection

The following resistance genes were identified:

1. Streptomycin *strA* and *strB*.
2. Tetracycline *tetA* and *tetB*.
3. Fluoroquinolone *gyrA, parC*
4. Sulfonamides *sul I*, *sul II*
5. Macrolides *mph(E), msr(E), erm 42*
6. Efflux pumps conferring antibiotic resistance *msr(E), adeB*
7. Aminoglycoside *aph(3')-VI, aacA4*
8. Trimethoprim *dhfr1*
9. *aadB,* confers tobramycin, gentamicin, and kanamycin resistance.

DNA sequence analysis was performed for both strands (forward and reverse primers) by the Immuno Gene Center (Erbil, Iraq). Online similarity searching was performed with the Basic Local Alignment Search Tool (BLAST) program in GenBank.

Results

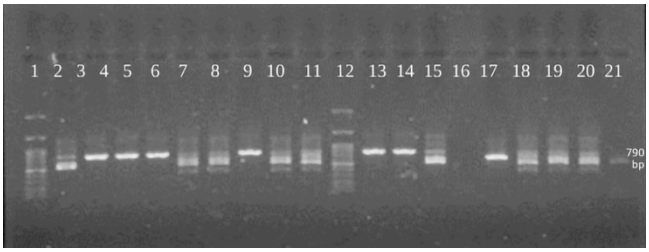
Identification of the clinical isolates

Among the 570 samples, 23 (4.04%) were identified as *A. baumannii* based on the colony morphology, biochemical test, and VITEK® 2 Compact system (Supplementary tables S3, S4, and S5).

Out of the 570 samples, 100 were COVID-19 patients' samples, and 13 (13%) strains of *A. baumannii* were isolated from these samples. All 13 isolates of COVID-19 patients were used in this study. In addition, two COVID-19 patients had coinfection with *Escherichia coli* and *Klebsiella pneumonia*.

The results of molecular identification by analysis of ITS fragments amplification and sequencing of 17 isolates with primers 1512F and 6R are shown in Figure.1. It was noted that 8 strains produced a single band on agarose gel electrophoresis; out of these 6 isolates were identified as *A. baumannii* by ITS sequence analysis (Supplementary table S6), and 2 isolates were identified as *Pseudomonas aeruginosa* (isolate14) and *Stenotrophomones maltophilia* novel strain (accession number OP422244, submitted to GenBank). The accession numbers are listed in Table 1.

Figure. 1. Amplification of *Acinetobacter* spp. DNA with 1512F and 6R primers and resolution of PCR products by 1.5% agarose gel electrophoresis (75 volts for 1 hour).



Well 1, 100-bp DNA ladder; followed by isolates 1, 2, 3, 4, 5, 6, 7, 8, 9, 10; well 12, 100-bp DNA ladder; well 13, isolate 11; well 14, isolate12; well 15, isolate 13; well 16, negative control; well 17, isolate14; well 18, isolate15; well 19, isolate 16; well 20, isolate 17; and well 21: *E. coli*.

Although 9 isolates resulted in multiple PCR products, as visualized through gel electrophoresis, the bacteria were identified as *A. baumannii* with the VITEK® 2 Compact system and β-lactamases genes sequencing analysis (Table 1 and Figure 1).

Biofilm formation

The bacterial isolates had variable responses to the production of biofilm; moderate biofilm formation was detected in 7 (41.2%) isolates, weak biofilm formation was detected in 6 (35.3%) isolates, and 3 (17.6%) isolates did not form biofilm. However, the number of non-biofilm-forming isolates was statically non-significant (Table 2).

Table 2. Biofilm formation in the bacterial isolates.

Strains	OD_{490} nm (mean ± SD)	Biofilm ability formation	*p*	t-test	Standard error	Statical significance
1	0.05033 ± 0.00107	No	0.9845	0.0206	0.016	non
2	0.07767 ± 0.0101	Weak	0.1794	1.6255	0.017	non
3	0.1877 ± 0.0535	Medium	0.0167	3.9588	0.035	significant
4	0.1347 ± 0.0335	Medium	0.0279	3.3749	0.025	significant
5	0.1433 ± 0.0117	Medium	0.0058	5.3742	0.017	very significant
6	0.1527 ± 0.0477	Medium	0.0321	3.2249	0.032	significant
7	0.08933 ± 0.0182	Weak	0.109	2.0553	0.019	non
8	0.05333 ± 0.00107	No	0.8453	0.2081	0.016	non
9	0.1443 ± 0.00786	Medium	0.0048	5.6725	0.017	very significant
10	0.063 ± 0.0037	Weak	0.4656	0.8057	0.016	non
11	0.1493 ± 0.0246	Medium	0.0097	4.6426	0.021	very significant
12	0.1207 ± 0.0252	Medium	0.0308	3.2701	0.022	significant
13	0.057 ± 0.00333	No	0.6863	0.4346	0.016	non
15	0.06633 ± 0.00324	Weak	0.3679	1.0142	0.016	non
17	0.1027 ± 0.00656	Weak	0.0327	3.2066	0.016	significant
(14) *P. aeruginosa*	0.073 ± 0.00733	Weak	0.2368	1.3903	0.017	non
Control	0.05 ± 0.00277					

OD ≤ 0.23 indicates strong; OD = 0.12 indicates medium; OD: 0.06–0.12 indicates weak; OD ≥ 0.06 indicates no. OD, optical density.

Plasmid detection

Plasmids, of size > 30 kb, were detected in 17 isolates (Supplementary Figure 1). The plasmids were screened by PCR for the presence of antibiotic resistance genes (Table 3, Supplementary Table 6 and Supplementary Figure 1)

Antibacterial susceptibility detection

The MIC of antibacterial agents was determined in isolates by VITEK® 2 compact system (BioMérieux, Marcy l'Etoile, France). All isolates showed sensitivity to colistin at MIC ≥ 0.5 μg/mL, while they showed high level of resistance to β-lactam and fluoroquinolone antibiotics (Table 4).

Detection of resistance genes on bacterial chromosome

Detection of β-lactamase (OXA carbapenemase) genes and sequencing

A single amplicon was observed for all (genomic) species in the detection of β-lactamases gene. All 15 isolates of *A. baumannii* were carbapenemase producers; however, bla_{OXA58} and bla_{SHV} genes were not detected in any isolate (Table 3 and Supplementary Table 6). The four β-lactamases genes (bla_{OXA23}-like genes, bla_{OXA-51}-like genes, bla_{TEM}, and bla_{NDM}) were detected in 4 (26.7%) isolates; three β-lactamases genes (bla_{OXA-51}-like gene, bla_{TEM} and bla_{NDM}) were detected in 5 (33.3%) isolates; two β-lactamases genes (bla_{TEM} and bla_{NDM}) were detected in 2 (13.3%) isolates; and one β-lactamases gene (bla_{TEM}) was detected in 2 (13.3%) isolates. Thus, most isolates harbored more than one carbapenemase production genes. The sequencing analysis of bla_{OXA23}-like genes and bla_{OXA51}-like gene amplicons revealed that all isolates had an identical nucleotide sequence, but sequencing bla_{TEM} and bla_{NDM} revealed nucleotide identity of *E. coli* and *Klebsiella pneumonia* plasmid genes. These new strains were named HK19 NDM, and HK22 TEM and submitted to GenBank under accession number OP572243 for bla_{NDM} and OP572244 for bla_{TEM}. Table1 lists all the detected β-lactamase genes, and Supplementary Table 6 includes genetic analyses of isolates. Interestingly, all tested β-lactamase genes were present in bacterial plasmids, except bla_{TEM}. Furthermore, the genes which were absent in the isolate's chromosome, existed in the isolate's plasmid (Table 1; Supplementary Table 6 and Supplementary Figures 2, 3, 4, and 5).

Table 3. Percentage of chromosomes and plasmids in *A. baumannii* isolates with β-lactamase gene.

Genes encoding β-lactamases	% Detection in isolates' chromosome DNA	% Detection in isolates' plasmid
bla_{OXA-51}*-like*[1]	62.5	18.8
bla_{OXA-23}*-like*[2]	33.3	46.7
bla_{OXA-58}*-like*[3]	0	20
bla_{TEM}	100	0
bla_{SHV}	0	26.7
bla_{NDM}	80	53.3

[1] Includes bla_{OXA-51}-, bla_{OXA-58}-, bla_{OXA-64}-, bla_{OXA-69}-, bla_{OXA-70}-, bla_{OXA-71}-, bla_{OXA-75}- and bla_{OXA-78}-like genes. [2] Includes bla_{OXA-23}-, bla_{OXA-27}-, and bla_{OXA-49}-like genes. [3] Includes bla_{OXA-58}- and bla_{OXA-96}-like genes

Detection of antibiotics resistance genes

Genes encoding efflux pump: *msr(E)*, *adeB*

Enhanced expression of efflux pump genes in *A. baumannii* confer resistance to aminoglycosides, quinolones, tetracyclines, and trimethoprim. The efflux pump gene *adeB* is a part of *AdeABC* pump that is essential for the antibiotic resistance mechanism in *A. baumannii*. The detection rate of the *adeB* gene was reported to be highest in clinical isolates of *A. baumannii* compared to other strains [22]. This amplicon was found in 14 (76.5%) isolates at 200 bp product size; plasmids of all isolates harbored this gene (Table 2). Based on ITS region sequencing, two isolates were identified as not *A. baumannii* (Supplementary Figure 6). However, *msrE* gene was not found in the chromosome of all isolates. The msr protein is responsible for resistance to macrolides [23] and 5 (33.3%) isolated plasmids contained the *msrE* gene (Supplementary Tables 7 and 8; Supplementary Figures 6 and 7).

Gene encoding macrolides, *mph(E), msr(E), erm 42*

The macrolides resistance genes, except the *msrE*, were absent, even in plasmids.

Genes encoding aminoglycosides *aadB*, *aph(3')-VI*, and *aacA4*

The *aadB* gene confers resistance to aminoglycosides such as tobramycin, gentamicin, and kanamycin. However, 17 isolates lacked the *aadB* gene. In addition, 4 (26.7%) isolates carried the *aacA4* gene, and 11 (73.3%) isolates carried the *aph(3')-VI* gene in their chromosomes. In the case of plasmids, 13 (86.7%) carried the *aacA4* gene and 100% carried the *aph(3')-VI* resistance gene (Supplementary Figures 8 and 9).

Gene encoding streptomycin resistance, *strA* and *strB*

The *strA* and *strB* genes coexisted in the chromosomes of 11 (73.3%) isolates and in all isolated plasmids (Supplementary Figure 10).

Gene encoding tetracycline resistance, *tetA* and *tetB*

Out of the 17 isolates analyzed by multiplex PCR, 1 (6.7%) carried *tetA* gene, and 11 (73.3%) carried *tetB*

gene, while 4 (26.7%) and 13 (93.3%) plasmids amplified the tetracycline resistance genes *tetA* and tetB respectively (Supplementary Figure 11).

Gene encoding fluoroquinolone resistance, *gyrA*, *parC*

Amplification of the *parC* and *gyrA* genes indicated that these two genes were prevalent in all 15 strains, in both chromosome and plasmid (Supplementary Figures 12 and 13).

Gene encoding sulfonamides, *sulI*, *sulII*

The sulfonamide resistance genes were found in both chromosomes and plasmids of isolates. *SulI* was detected in 11 (73.4%) isolates; *SulII* was present in 6 (40%) isolates; and the two genes were detected in all (100%) plasmids of isolates (Supplementary Figure 14).

Gene encoding trimethoprim, *dhfr1*

Interestingly, the trimethoprim gene *dhfr1* was encoded by only 5 (33.3%) plasmids isolated, whilst this gene was absent in the chromosomes of all isolates (Supplementary Figure 15).

Overall, although the macrolide and trimethoprim resistance genes were detected in fewer isolates, the strains harbored several antibiotic resistance genes that were acquired from other bacterial genera (Supplementary Table 9).

Discussion

The goal of this study was to determine the resistance characteristics in *A. baumannii*, by taking a "snapshot" of this complex bacteria during a pandemic. Based on this study, it is obvious that there is a significant outbreak of multidrug resistant *A. baumannii* infections in hospitals.

A significant portion of the isolates were from COVID-19 patients (13%) and this may indicate the nature and severity of this disease among the patients. Most of the COVID-19 patients were in intensive care units (ICUs). Only 7 (1.23%) isolates were from urinary tract infections (Supplementary Tables 10 and 11).

Studies conducted in the pre-COVID-19 period, such as the study during the period 2018-2019 in Duhok, Iraq reported that 6.8 % *A. baumannii* were

Table 4. Antibiotics susceptibility testing by VITEK® 2 Compact system on *A. baumannii* isolates.

Antimicrobial agent	MIC (µg/mL)		No. (%) of isolates tested		
	Inhibited zone diameter	No. (%) of isolates	Susceptible	Intermediate	Resistant
Piperacillin	≥ 128	17 (100)	0	0	17 (100)
Piperacillin/Tazobactam	≥ 128	17 (100)	0	0	17 (100)
Ceftazidime	≥ 64	15 (88.2)	0	0	17 (100)
	≥ 32	2 (11.8)			
Cefepime	1	1(5.9)	1 (5.9)	0	16 (94.1)
	16	1(5.9)			
	≥ 32	12 (70.6)			
	≥ 64	3 (17.6)			
Imipenem	1	1(5.9)	1 (5.9)	0	16 (94.1)
	≥ 16	16(94.1)			
Meropenem	≤ 10	2 (11.8)	2 (11.8)	0	15 (88.2)
	≥ 16	15(88.2)			
Gentamicin	≤ 1	6(35.3)	6 (35.3)	1(5.9)	11(64.7)
	8	1(5.9)			
	≥ 16	11(64.7)			
Netilmicin	≥ 32	7 (53.8)	5 (38.5)	1(7.7)	7 (53.8)
	16	1(7.7)			
	4	4 (30.8)			
	2	1(7.7)			
Tobramycin	≥ 16	6 (42.9)	8 (57.1)	0	6 (42.9)
	4	1 (7.1)			
	2	1 (7.1)			
	≤ 1	6 (42.9)			
Ciprofloxacin	≥ 4	16 (94.1)	1 (5.9)	0	16 (94.1)
Levofloxacin	≥ 8	17 (100)	0	0	17 (100)
Tetracycline	≥ 16	13 (92.9)	1 (7.1)	0	13 (92.9)
	4	1 (7.1)			
Tigecycline	≥ 8	1 (7.7)	2 (15.4)	8 (61.5)	1 (7.7)
	4	8 (61.5)			
	2	3 (23.1)			
	≥ 0.5	1 (7.7)			
Colstin	≥ 0.5	17 (100)	17 (100)	0	0
Trimethoprim/	≥ 320	13 (76.5)	3 (17.6)	0	14 (82.4)
Sulfamethaxole	80	1 (5.9)			
	≥ 20	3 (17.6)			

MIC: minimum inhibitory concentration.

isolated from burn and sputum samples [24]. In a similar study in Baghdad, *A. baumannii* was found in 12.82% of urine samples, and 11.94% of sputum samples [25]. When we compare these findings to our study, we note an increase in the number of isolates after the COVID-19 pandemic.

Our findings can be categorized into three levels: 1) characteristics of the isolates related to the pathogenesis; 2) antibiotics resistance and emergence of epidemic; and 3) sequencing of bacterial genes leading to investigating altered characteristics of the isolates.

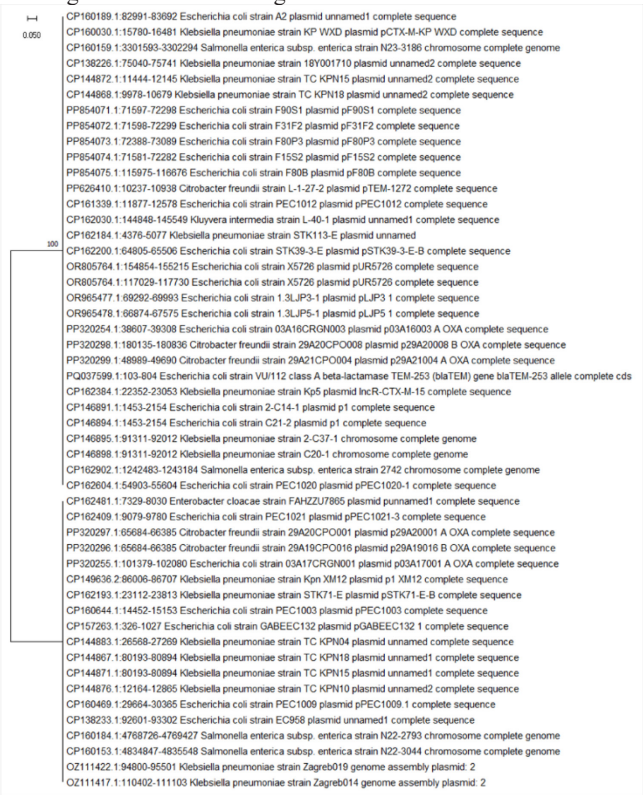
Biofilm formation was detected in 41.2% isolates. Despite the presence of several resistance genes in all isolates, there was no significant biofilm formation in some. Several studies in the past have found that *A. baumannii* multidrug resistance is associated with biofilm formation [26-28]. Furthermore, plasmids of size > 3 kbp were extracted from all isolates, indicating the same plasmid lineage. Bioinformatics analysis concluded that only 34.6% of *A. baumannii* plasmids possessed multidrug resistance genes and belonged to 14 plasmid lineages out of the 21 *A. baumannii* plasmid lineages described previously [29]. Usually, the small plasmid size in *A. baumannii* is difficult to transfer, and alternative mechanisms for transformation are used. Moreover, in the > 20 kb plasmids resistance genes are within mobile genetic elements such as transposons and integrons, or excised phage. The plethora of antibiotic resistance genes on plasmids promotes survival of *A. baumannii* in clinical environment [30].

A second striking finding of this study is the large number of antibiotic resistance genes identified in the isolates. All isolates had eight or more resistance determinants. Our genetic analysis revealed that the bla_{TEM} were found in all of the chromosome strains. More significantly, we also detected the presence of the bla_{NDM} in 80%, bla_{OXA-51}-like gene in 62.5 %, and bla_{OXA-23}-like gene in 33.3% isolates. The bla_{OXA-58}-like gene and bla_{SHV} were not detected. In the case of plasmids, resistance genes bla_{TEM}, bla_{NDM}, bla_{OXA-51}-like gene, bla_{OXA-23}-like gene, bla_{OXA-58}-like gene, and bla_{SHV} were detected in 0%, 53.3%, 18.8%, 46.7%, 20% and 26.7% respectively. Thus, the bla_{TEM} gene was disseminated in the isolates' chromosome and contributed to piperacillin, piperacillin/tazobactam, and ceftazidime resistance in 100%; cefepime and imipenem resistance in 94%; and meropenem resistance in 88.2% of the carbapenem-resistant isolates in this collection.

This analysis contrasted to previous studies [31-40], where intrinsic and chromosomally located OXA-51-like β-lactamases; and acquired OXA-23-like and OXA-58-like β-lactamases were found in carbapenem-resistant *A. baumannii* isolates. bla_{OXA-23} was found to be responsible for imipenem resistance in the majority of strains and added to resistance mechanisms by horizontal gene transfer [33]. In addition to the most common carbapenem-resistant *Acinetobacter baumannii* mechanisms is the presence of oxacillinases OXA-23, OXA-58, and NDM-like β-lactamases [38]. Carbapenem-resistant *Acinetobacter baumannii* class D in this study belonged to international clonal lineage II (ICL-II), and most strains were isolated from patients in intensive care units (ICUs) [34].

New nucleotide sequences of β-lactamase genes were identified in different isolates (Table 1). These genes sequences, located in the bla_{NDM} and bla_{TEM} genes were acquired from the Enterobacteriaceae genera by transferring mobile genetics elements in the ICU patients. These were identified as coinfections and may act as a potential link to higher levels of carbapenem resistance, except for colistin (Figure 1 and 2). The other β-lactamase gene sequences included in the analysis of this study were identified in the NCBI gene bank after COVID-19 outbreaks; this may be an

Figure 2. Distance tree of the new bla_{NDM} gene of *A. baumannii* (Genebank accession number OP572243) which was isolated from COVID-19 ICU patients demonstrating the origin of the new gene from horizontal gene transfer.



indication that the disease outbreak is promoting an epidemic of resistant *A. baumannii*. In addition, the identification of these β-lactamase genes in the NCBI gene bank during the COVID-19 pandemic, suggests a potential for global dissemination. The sequence analysis has also shown that the new strain *Stenotrophomones maltophilia* strain HK in our study has *A. baumannii* β -lactamase, $bla_{oxa\text{-}51}$-like genes and bla_{NDM}.

Most importantly, carbapenemase producing *Acinetobacter* spp. were disseminated in many Erbil hospitals. Interestingly, 100% of isolates from the 5 hospitals produced β-lactamase. The study also noted that extended-spectrum β-lactamases (ESBLs) producing isolates including bla_{TEM} and bla_{SHV} were much more prevalent (100%) than those with OXA β-lactamases producing bla_{TEM} and bla_{SHV} and confer resistance to cephalosporins (cefotaxime, ceftriaxone, ceftazidime, and aztreonam). More importantly, the new bla_{TEM} carrying isolates were detected (Table 1). bla_{TEM} and bla_{SHV} that are encoded frequently by plasmids carry resistance to other antibiotic classes [36] contributing to the high numbers of different resistance genes in isolated plasmids in this study. The bla_{TEM} was encoded by 100% of bacterial chromosomes, whereas bla_{SHV} was encoded by 26.7% of plasmid. Consequently, the role of plasmids is essential in gene transfer to bacterial chromosomes to increase *A. baumannii* pathogenicity.

It is notable that these isolates carried resistance genes *gyrA*, and *parC*, that confer resistance to fluoroquinolone, on both plasmids and chromosomes, which exhibited phenotypically as 94% resistance to ciprofloxacin and 100% resistance to levofloxacin.

On the other hand, the resistance to gentamycin, netilmicin, and tobramycin were 64.7%, 53.8%, and 42.9% respectively. Interestingly, the resistance genes of this group were encoded 100% in plasmids and 73.3% in chromosomes; except *aadB* which was not encoded by the bacteria. *aadB* is responsible for tobramycin resistance [37].

Efflux pump gene *aacA4* which is related to aminoglycosides resistance was encoded 86.7% in plasmid and 26.7% in the chromosome; whereas the *AdeB* which is involved in the resistance to other antibiotics such as tigecycline lactams, chloramphenicol, erythromycin, and tetracycline was encoded 100% in plasmid and 86.7% in the chromosome.

Up to 92% tetracycline resistance was recorded and the genes were encoded by the chromosomes (6.7% *TetA*, and 73.3% *TetB*) and by the plasmids (26.7% *TetA*, and 93.3% *TetB*). The resistance genes for both macrolide and trimethoprim were the least encoded genes at 33.3% on plasmids; therefore, these antibiotics would be effective on the majority of isolates. The combination of trimethoprim with sulfonamide resistance was 82.4%. This might be due to the presence of the resistance genes (*Sul1* and *Sul2* in 100% plasmids, and 73.3% *Sul1* and 40% *Sul2* in chromosome).

Apart from the role of metabolic pathways that are related to bacteria survival and pathogenicity (Table 4), we demonstrated that 93.8% of the isolates metabolized D-cellobiose, D-glucose, D-mannose, malonate, L-lactate alkalinication, and succinate alkalinication; and 100% isolates metabolized coumarate. These metabolic pathways are involved in bacterial virulence determination [38]. Interestingly, utilization of the sodium citrate and glutamyl arylamidace pNA was at 87.5% arylamidase activities, specific for basic amino acid hydrolysis, and host tyrosine hydrolysis by specific bacterial virulence factors [45,46]. Essential bacterial virulence factors activate arylamidase to hydrolyze tyrosine [39,40]. Interestingly, 87.5% of the isolates utilized sodium citrate and glutamyl arylamidase pNA. Significantly, sodium citrate and malonate inhibited bacterial biofilm formation and decreased virulence factors [41,42]. On the other hand, utilization of L-

Figure 3. Distance tree of the new bla_{TEM} gene of *A. baumannii* (Genebank accession number OP572244) which was isolated from COVID-19 ICU patients demonstrating the origin of the new gene from horizontal gene transfer.

proline arylamidase was 18.8%, tyrocine arylamidase 81.3%, urease 43.8%, L−histidine assimilation and L−malate assimilation were 37.5 %, Ellman was 12.5 %, and L−lactate assimilation was 56.3%. *A. baumannii* reduced catabolism of mannitol and glutamate to survive in the dry hospital environment; this shift in metabolism pathway decreases growth and makes it unable to metabolize various amino acids by gene mutation [43].

Our study demonstrated the metabolic role of our isolates in invading the immune system and causing inflammation with sepsis through phenylacetic acid catabolism pathway using coumarate enzyme. The role of the coumarate (coenzyme A ligase) in evading host immune system has been previously demonstrated [44,45]. The phenylacetic acid catabolism pathway regulates all isolates to evade host immunity through the coumarate enzyme (coenzyme A ligase) causes inflammation with sepsis [44,45]. Whenever the neutrophils are activated against the pathogen, bacteria produce reactive nitrogen species, and the pathogens shift metabolic pathways to survive, thus increasing the risk of invasion [46-48].

Our comprehensive analysis is the first study to link the multidrug resistance phenotypes and genetic determinants of resistance to β-lactams, aminoglycosides, fluoroquinolone, tetracycline, sulfonamide, trimethoprim, and macrolides in *A. baumannii* in Erbil, Iraq. Many of these *A. baumannii* isolates serve as reservoirs for antibiotic-resistance genes that were transmitted to other pathogens by plasmids.

Conclusions

Our study identified the prevalent bla_{TEM}, and new bla_{TEM} and bla_{NDM}. Additionally, this study demonstrated the role of the strain's metabolic pathway in isolate characterizations and resistance determination; thus, metabolism pathways can be an attractive therapeutic approach. The fermentation pathway increases gene transfer and integration of bacteria [49-51]. The diversity of the new finding's resistance determinants and the potential for broad distribution of these strains could alter the worldwide epidemiology of *A. baumannii*-related diseases.

Acknowledgements

The authors thank the Science and Health Research Center at Koya University and Salahaddin University Research Center of Erbil, Iraq.

Ethics approval

Approval was obtained from the ethics committee of Koya University. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

Authors' contributions

Both authors (HMH and HTS) contributed to the study conception and design, material preparation, data collection, and analysis. HTS wrote the first draft of the manuscript; both authors read and approved the final manuscript.

Conflict of interests

No conflict of interests is declared.

References

1. Peacock SJ, Parkhill J, Brown NM (2018) Changing the paradigm for hospital outbreak detection by leading with genomic surveillance of nosocomial pathogens. Microbiology 164: 1213–1219. doi: 10.1099/mic.0.000700.
2. World Health Organization (2017) Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. Geneva. Available: https://www.doherty.edu.au/news-events/news/who-global-priority-pathogens-list-of-antibiotic-resistant-bacteria. Accessed: 17 May 2024.
3. Ripa M, Galli L, Poli A, Oltolini C, Spagnuolo V, Mastrangelo A, Muccini C, Monti G, De Luca G, Landoni G, Dagna L, Clementi M, Rovere Querini P, Ciceri F, Tresoldi M, Lazzarin A, Zangrillo A, Scarpellini P, Castagna A, COVID-BioB study group (2021) Secondary infections in patients hospitalized with COVID-19: incidence and predictive factors. Clin Microbiol Infect 27: 451–457. doi: 10.1016/j.cmi.2020.10.021.
4. Giammanco A, Calà C, Fasciana T, Dowzicky MJ (2017) Global assessment of the activity of tigecycline against multidrug-resistant Gram-negative pathogens between 2004 and 2014 as part of the tigecycline evaluation and surveillance trial. mSphere 2: e00310–16. doi: 10.1128/mSphere.00310-16.
5. Nutman A, Lerner A, Schwartz D, Carmeli Y (2016) Evaluation of carriage and environmental contamination by carbapenem-resistant *Acinetobacter baumannii*. Clin Microbiol Infect 22: 949.e5–949.e7. doi: 10.1016/j.cmi.2016.08.020.
6. Fan L, Wang Z, Wang Q, Xiong Z, Xu Y, Li D, Fan L, Zhou S (2018) Increasing rates of *Acinetobacter baumannii* infection and resistance in an oncology department. J Cancer Res Ther14: 68. doi: 10.4103/jcrt.JCRT_737_17.
7. Rangel K, Chagas TPG, De-Simone SG (2021) *Acinetobacter baumannii* infections in times of COVID-19 pandemic. Pathogens 10: 1006. doi: 10.3390/pathogens10081006.
8. Wang Z, Yang B, Li Q, Wen L, Zhang R (2020) Clinical features of 69 cases with coronavirus disease 2019 in Wuhan, China. Clin Infect Dis 71: 769–777. doi: 10.1093/cid/ciaa272.
9. Contou D, Claudinon A, Pajot O, Micaëlo M, Longuet Flandre P, Dubert M, Cally R, Logre E, Fraissé M, Mentec H, Plantefève G (2020) Bacterial and viral co-infections in patients with severe SARS-CoV-2 pneumonia admitted to a French ICU. Ann Intensive Care 10: 119. doi: 10.1186/s13613-020-00736-x.
10. Ripa M, Galli L, Poli A, Oltolini C, Spagnuolo V, Mastrangelo A, Muccini C, Monti G, De Luca G, Landoni G, Dagna L, Clementi M, Rovere Querini P, Ciceri F, Tresoldi M, Lazzarin

A, Zangrillo A, Scarpellini P, Castagna A (2021) Secondary infections in patients hospitalized with COVID-19: incidence and predictive factors. Clin Microbiol Infect 27: 451–457. doi: 10.1016/j.cmi.2020.10.021.
11. Morris FC, Dexter C, Kostoulias X, Uddin MI, Peleg AY (2019) The mechanisms of disease caused by *Acinetobacter baumannii*. Front Microbiol 10: 1601. doi: 10.3389/fmicb.2019.01601.
12. Mariana P, Francisco MA, Afonso Luis B, (2016) Mobile genetic elements related to carbapenem resistance in *Acinetobacter baumannii*. Braz J Microbiol 47: 785–792. doi: 10.1016/j.bjm.2016.06.005.
13. Walther-Rasmussen J, Høiby N (2006) OXA-type carbapenemases. J Antimicrob Chemother 57: 373–383. doi: 10.1093/jac/dki482.
14. Abdi SN, Ghotaslou R, Ganbarov K, Mobed A, Tanomand A, Yousefi M, Asgharzadeh M, Kafil HS (2020) *Acinetobacter baumannii* efflux pumps and antibiotic resistance. Infect Drug Resist 12: 423–434. doi: 10.2147/IDR.S228089.
15. Xu CF, Bilya SR, Xu W (2019) adeABC efflux gene in *Acinetobacter baumannii*. New Microbes and New Infect 30: 100549. doi: 10.1016/j.nmni.2019.100549.
16. Kyriakidis I, Vasileiou E, Pana ZD, Tragiannidis A (2021) *Acinetobacter baumannii* antibiotic resistance mechanisms. Pathogens 10: 373. doi: 10.3390/pathogens10030373.
17. Tille Patricia M (2014) Bailey & Scott's Diagnostic Microbiology, 15th edition, Missouri: Elsevier 370 p.
18. Chang HC, Wei YF, Dijkshoorn L, Vaneechoutte M, Tang CT, Chang TC (2005) Species-level identification of isolates of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex by sequence analysis of the 16S-23S rRNA gene spacer region. J Clin Microbiol 43: 1632–1639. doi: 10.1128/JCM.43.4.1632-1639.2005.
19. O'Toole A, Kolter R (1998) Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. Molecular Microbiology 30: 295–304. doi: 10.1046/j.1365-2958.1998.01062.x.
20. Subhi HT (2018) Activity of iron oxide nanoparticles-chitosan composite on bacterial biofilm formation. ICPAS 2018: 7–10. doi: 10.14500/icpas2018.mim104.
21. Bardbari AM, Arabestani MR, Karami M, Keramat F, Alikhani MY, Bagheri KP (2017) Correlation between ability of biofilm formation with their responsible genes and MDR patterns in clinical and environmental *Acinetobacter baumannii* isolates. Microb Pathog 108: 122–128. doi: 10.1016/j.micpath.2017.04.039
22. Xu C, Bilya SR, Xu W (2019) *adeABC* efflux gene in *Acinetobacter baumannii*. New Microbes New Infect 30: 100549. doi: 10.1016/j.nmni.2019.100549.
23. Huang L, Wu C, Gao H, Xu C, Dai M, Huang L, Hao H, Wang X, Cheng G (2022) Bacterial multidrug efflux pumps at the frontline of antimicrobial resistance: an overview. Antibiotics 11: 520. doi: 10.3390/antibiotics11040520.
24. Abdullah ZH, Merza NS (2019). Phenotypic and molecular detection of *Acinetobacter baumannii* isolated from patients in Duhok city-Iraq. Science Journal of University of Zakho 7: 132–137. doi: 10.25271/sjuoz.2019.7.4.644.
25. AL-Mousawi HT, Nader AL- Taee MI, AL-Hajjar QN (2018) Evaluation of biofilm formation capacity of *Acinetobacter baumannii* isolated from clinical samples in Baghdad hospitals using phenotypic methods. Iraqi Journal of Biotechnology 17: 47–60.
26. Longo F, Vuotto C, Donelli G (2014) Biofilm formation in *Acinetobacter baumannii*. New Microbiol 37: 119–127.
27. Yang CH, Su PW, Moi SH, Chuang LY (2019) Biofilm formation in *Acinetobacter baumannii*: genotype-phenotype correlation. Molecules 24: 1849. doi: 10.3390/molecules24101849.
28. Upmanyu K, Haq QMR, Singh R (2022) Factors mediating *Acinetobacter baumannii* biofilm formation: opportunities for developing therapeutics, Curr Res Microb Sci 3: 100131. doi: 10.1016/j.crmicr.2022.100131.
29. Salgado-Camargo AD, Castro-Jaimes S, Gutierrez-Rios R-M, Lozano LF, Altamirano-Pacheco L, Silva-Sanchez J, Pérez-Oseguera Á, Volkow P, Castillo-Ramírez S and Cevallos MA (2020) Structure and evolution of *Acinetobacter baumannii* plasmids. Front Microbiol 11: 1283. doi: 10.3389/fmicb.2020.01283.
30. Brovedan MA, Cameranesi MM, Limansky AS, Morán-Barrio J, Marchiaro P, Repizo GD (2020) What do we know about plasmids carried by members of the *Acinetobacter* genus? World J Microbiol Biotechnol 36: 109. doi: 10.1007/s11274-020-02890-7.
31. Poirel L, Naas T, Nordmann P (2010) Diversity, epidemiology, and genetics of class D beta-lactamases. Antimicrob Agents Chemother 54: 24–38. doi: 10.1128/aac.01512-08
32. Nguyen M, Joshi SG (2021) Carbapenem resistance in *Acinetobacter baumannii*, and their importance in hospital-acquired infections: a scientific review. J Appl Microbiol 131: 2715–2738. doi: 10.1111/jam.15130.
33. Huang XZ, Chahine MA, Frye JG, Cash DM, Lesho EP, Craft DW, Lindler LE, Nikolich MP (2012) Molecular analysis of imipenem-resistant *Acinetobacter baumannii* isolated from US service members wounded in Iraq, 2003-2008. Epidemiol Infect 140: 2302–2307. doi: 10.1017/S0950268811002871.
34. Minandri F, D'Arezzo S, Antunes LC, Pourcel C, Principe L, Petrosillo N, Visca P (2012) Evidence of diversity among epidemiologically related carbapenemase-producing *Acinetobacter baumannii* strains belonging to international clonal lineage II. J Clin Microbiol 50: 590–597. doi: 10.1128/JCM.05555-11.
35. Yousefi Nojookambari N, Sadredinamin M, Dehbanipour R, Ghalavand Z, Eslami G, Vaezjalali M, Nikmanesh B, Yazdansetad S (2021) Prevalence of β-lactamase-encoding genes and molecular typing of *Acinetobacter baumannii* isolates carrying carbapenemase OXA-24 in children. Ann Clin Microbiol Antimicrob 20: 75. doi: 10.1186/s12941-021-00480-5.
36. Paterson DL, Bonomo RA (2005) Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 18: 657–686. doi: 10.1128/CMR.18.4.657-686.2005.
37. Hujer KM, Hujer A M, Hulten EA, Bajaksouzian S, Adams JM, Donskey CJ (2006) Analysis of antibiotic resistance genes in multidrug-resistant *Acinetobacter* sp. isolates from military and civilian patients treated at the Walter Reed Army Medical Center. Antimicrob Agents Chemother 50: 4114–4123. doi: 10.1128/AAC.00778-06.
38. Wu MC, Chen YC, Lin TL, Hsieh PF, Wang JT (2012) Cellobiose-specific phosphotransferase system of *Klebsiella pneumoniae* and its importance in biofilm formation and virulence. Infect Immun 80: 2464–2472. doi: 10.1128/IAI.06247-11.
39. Fukasawa K, Hiraoka BY, Fukasawa KM, Harada M (1982) Arylamidase activities specific for proline, tyrosine, and basic

amino acid residues in some oral bacteria. J Dent Res 61: 818–820. doi: 10.1177/00220345820610063501.
40. Bliska JB, Guan K, Dixon JE, Falkow S (1991) Tyrosine phosphate hydrolysis of host proteins by an essential yersinia virulence determinant. Proc Natl Acad Sci USA 88: 1187–1191. doi: 10.1073/pnas.88.4.1187.
41. Khayat MT, Ibrahim TS, Khayyat AN, Alharbi M, Shaldam MA, Mohammad KA, Khafagy ES, El-Damasy DA, Hegazy W, Abbas HA (2022) Sodium citrate alleviates virulence in *Pseudomonas aeruginosa*. Microorganisms 10: 1046. doi: 10.3390/microorganisms10051046.
42. Elmassry, MM, Bisht, K, Colmer-Hamood, JA, Wakeman, CA, San Francisco, MJ, Hamood, AN (2021) Malonate utilization by *Pseudomonas aeruginosa* affects quorum-sensing and virulence and leads to formation of mineralized biofilm-like structures. Mol Microbiol 116: 516–537. doi: 10.1111/mmi.14729.
43. König P, Averhoff B, Müller V (2021) K+ and its role in virulence of *Acinetobacter baumannii*, Int J Med Microbiol 311: 151516. doi: 10.1016/j.ijmm.2021.151516.
44. Kaneko M, Ohnishi Y, Horinouchi S (2003) Cinnamate: coenzyme A ligase from the filamentous bacterium *Streptomyces coelicolor* A3(2). J Bacteriol 185: 20–27. doi: 10.1128/JB.185.1.20-27.2003.
45. Bhuiyan MS, Ellett F, Murray GL, Kostoulias X, Cerqueira GM, Schulze KE, Mahamad Maifiah MH, Li J, Creek DJ, Lieschke GJ, Peleg AY (2016) *Acinetobacter baumannii* phenylacetic acid metabolism influences infection outcome through a direct effect on neutrophil chemotaxis. Proc Natl Acad Sci USA 113: 9599–9604. doi: 10.1073/pnas.1523116113.
46. Spahich NA, Vitko NP, Thurlow LR, Temple B, Richardson AR (2016) *Staphylococcus aureus* lactate- and malate-quinone oxidoreductases contribute to nitric oxide resistance and virulence. Mol Microbiol 100: 759–773. doi: 10.1111/mmi.13347.
47. Poladyan A, Trchounian K, Tadevosyan L, Trchounian A (2008) Effects of Ellman's reagent and other thiol compounds on ion transport and ATPase activity in anaerobically grown *Escherichia coli* cells. Biochem. Moscow Suppl A2: 1–7. doi: 10.1134/S1990747808010017.
48. Richardson AR, Payne EC, Younger N, Karlinsey JE, Thomas VC, Becker LA, Navarre WW, Castor ME, Libby SJ, Fang FC (2011) Multiple targets of nitric oxide in the tricarboxylic acid cycle of *Salmonella enterica* serovar *typhimurium*. Cell Host Microbe 10: 33–43. doi: 10.1016/j.chom.2011.06.004.
49. Philipps G, de Vries S, Jennewein S (2019) Development of a metabolic pathway transfer and genomic integration system for the syngas-fermenting bacterium *Clostridium ljungdahlii*. Biotechnol Biofuels 8: 112. doi: 10.1186/s13068-019-1448-1.
50. Stasiak G, Mazur A, Wielbo J, Marczak M, Zebracki K, Koper P, Skorupska A (2014) Functional relationships between plasmids and their significance for metabolism and symbiotic performance of *Rhizobium leguminosarum* bv. *trifolii*. J Appl Genet 55: 515–527. doi: 10.1007/s13353-014-0220-2.
51. San Millan A, Toll-Riera M, Qi Q, Betts A, Hopkinson RJ, McCullagh J, MacLean RC (2018) Integrative analysis of fitness and metabolic effects of plasmids in *Pseudomonas aeruginosa* PAO1. ISME J 12: 3014–3024. doi: 10.1038/s41396-018-0224-8.
52. Noppe-Leclercq I, Wallet F, Haentjens S, Courcol R, Simonet M (1999) PCR detection of aminoglycoside resistance genes: a rapid molecular typing method for *Acinetobacter baumannii*. Res Microbiol 150: 317–322. doi: 10.1016/S0923-2508(99)80057-6.
53. Modarresi F, Azizi O, Shakibaie MR, Motamedifar M, Valibeigi B, Mansouri S (2015) Effect of iron on expression of efflux pump (adeABC) and quorum sensing (luxI, luxR) genes in clinical isolates of *Acinetobacter baumannii*. APMIS 123: 959–968. doi: 10.1111/apm.12455.
54. Cho S, Nguyen HAT, McDonald JM, Woodley TA, Hiott LM, Barrett JB, Jackson CR, Frye JG (2019) Genetic characterization of antimicrobial-resistant *Escherichia coli* isolated from a mixed-use watershed in northeast Georgia, USA. Int J Environ Res Public Health 16: 3761. doi: 10.3390/ijerph16193761.
55. Rose S, Desmolaize B, Jaju P, Wilhelm C, Warrass R, Douthwaite S (2012) Multiplex PCR to identify macrolide resistance determinants in *Mannheimia haemolytica* and *Pasteurella multocida*. Antimicrob Agents Chemother 56: 3664–3669. doi: 10.1128/AAC.00266-12.
56. Brunelle BW, Bearson BL, Bearson SM (2015) Chloramphenicol and tetracycline decrease motility and increase invasion and attachment gene expression in specific isolates of multidrug-resistant *Salmonella enterica* serovar *Typhimurium*. Front Microbiol 5: 801. doi: 10.3389/fmicb.2014.00801.
57. Kerrn MB, Klemmensen T, Frimodt-Møller N, Espersen F (2002) Susceptibility of Danish *Escherichia coli* strains isolated from urinary tract infections and bacteraemia, and distribution of *sul* genes conferring sulphonamide resistance. J Antimicrob Chemother 50: 513–516. doi: 10.1093/jac/dkf164.
58. Aminov RI, Chee-Sanford JC, Garrigues N, Teferedegne B, Krapac IJ, White BA, Mackie RI (2002) Development, validation, and application of PCR primers for detection of tetracycline efflux genes of Gram-negative bacteria. Appl Environ Microbiol 68:1786–1793. doi: 10.1128/AEM.68.4.1786-1793.2002.
59. Stine OC, Johnson JA, Keefer-Norris A, Perry KL (2007) Widespread distribution of tetracycline resistance genes in a confined animal feeding facility. Int J Antimicrob Agents 29: 348–352. doi: 10.1016/j.ijantimicag.2006.11.015.
60. Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL (2006) Identification of *Acinetobacter baumannii* by detection of the *blaOXA-51*-like carbapenemase gene intrinsic to this species. J Clin Microbiol 44: 2974–2976. doi: 10.1128/JCM.01021-06.

Corresponding author

Hanan Tariq Subhi, PhD.
Department of Biology, Faculty of Science and Health, Koya University, Danielle Mitterrand Boulevard,
Koysinjaq, Koya KOY45, Kurdistan Region – F.R. Iraq
Tel: +964 7702491428
Email: hanan.baker@koyauniversity.org

Annex – Supplementary Items

Supplementary Table 1. Primers, target genes, and size of the amplified products.

Primer	Target genes	Sequence (5-3)	Amplicon size (bp)	References
1512F 6R	16S-23S rRNA	GTCGTAACAAGGTAGCCGTA GGGTTYCCCCRTTCRGAAAT3	790	[18]
OXA 51 F OXA 51 R	*bla*$_{OXA}$51	TAATGCTTTGATCGGCCTTG TGGATTGCACTTCATCTTGG	320	[60]
OXA 58 F OXA58 R	*bla*$_{OXA-58}$, *bla*$_{OXA-96}$	ATGAAATTATTAAAAATATTGAGTTTAG TTATAAATAATGAAAAACACCCAAC	≈500	
OXA SET B F OXA SET B R OXA SET C F OXA SET C R	*blaOXA-23, blaOXA-27, blaOXA-49* *blaOXA-51, blaOXA-58, blaOXA-64, blaOXA-69, blaOXA-70, blaOXA-71, blaOXA-75, and blaOXA-78*	TCTGGTTGTACGGTTCAGC AGTCTTTCCAAAAATTTTG ACAGAARTATTTAAGTGGG GGTCTACAKCCMWTCCCCA	580 350	[37]
NDM F NDM R	*bla*$_{NDM}$	GGTTTGGCGATCTGGTTTTC CGGAATGGCTCATCACGATC	590	[31]
SHV F SHV R	*bla*$_{SHV}$	ATGCGTTATATTCGCCTGTG TGCTTTGTTATTCGGGCCAA	≈200	
TEM F TEM R	*bla*$_{TEM}$	AAACGCTGGTGAAAGTA AGCGATCTGTCTAT	720	[37]
aacA4 F aacA4 R	*aacA4*	ATGACTGAGCATGACCTTGCG TTAGGCATCACTGCGTGTTCG	530	
aadB F aadB R	*aadB*	ATGGACACAACGCAGGTCGC TTAGGCCGCATATCGCGACC	----------	
aph(3')-VI F aph(3')-VI R	*aph(3')-VI*	CGGAAACAGCGTTTTAGA TTCCTTTTGTCAGGTC	716	[52]
AdeB F AdeB R	*adeB*	CTTGCATTTACGTGTGGTGT GCTTTTCTACTGCACCCAAA	200	[53]
gyrA F gyrA R	*gyrA*	AAATCTGCCCGTGTCGTTGGT GCCATACCTACGGCGATACC	350	[37]
ParC F parC R	*parC*	AAACCTGTTCAGCGCCGCATT AAAGTTGTCTTGCCATTCA	327	
dhfr1 F dhfr1 R	*dhfr1*	CGGTCGTAACACGTTCAAGT CTGGGGATTTCAGGAAAGTA	220	[54]
msr(E) F msr(E) R	*msr(E)*	TATAGCGACTTTAGCGCCAA GCCGTAGAATATGAGCTGAT	400	
mph(E) F mph(E) R	*mph(E)*	ATGCCCAGCATATAAATCGC ATATGGACAAAGATAGCCCG	271	[55]
erm(42) F erm(42) R	*erm(42)*	TGCACCATCTTACAAGGAGT CATGCCTGTCTTCAAGGTTT	173	
strA F strA R	*strA*	GGTAAGAAGTCGGGATTGAC CACAGCCTATCGGTTGATC	165	[56]
strB F strB R	*strB*	CGCCATACCAGATAGTCG CTTTTCCAGCCTCGTTTG	600	
sul I F sul I R	*sul I*	CGGCGTGGGCTACCTGAACG GCCGATCGCGTGAAGTTCCG	433	[57]
sul II F sul II R	*sul II*	GCGCTCAAGGCAGATGGCATT GCCACTGCGCCGTTACCACC	293	
tetA F tetA R	*tetA*	GCGCGATCTGGTTCACTCG AGTCGACAGYRGCGCCGGC	164	[58]
tetB F tetB R	*tetB*	CGTTTGCTTTCAGGGATCA ACCATCATGCTATTCCATCC	437	[59]

Supplementary Table 2. Thermal cycling parameters for each primer pair.

Primer	Initial denaturation			Denaturation		Annealing		Extension		Final extension	
	No. of cycles	Temp. °C	Time (min)	Temp. °C	Time (sec)	Temp. °C	Time (sec)	Temp. °C	Time (sec)	Temp. °C	Time (min)
16s 23s rRNA	30	94	2	94	60	51	60	72	60	72	7
OXA 51	30	95	3	95	45	57	40	72	60	72	5
OXA 58	30	94	10	94	30	53	60	72	50	72	5
OXA SET B	30	94	10	94	30	51	60	72	50	72	5
OXA SET C	30	94	10	94	30	47	60	72	50	72	5
TEM	30	94	10	94	30	45	60	72	50	72	5
SHV	30	94	10	94	30	60	60	72	50	72	5
NDM	30	95	5	95	30	52	30	72	45	72	5
aacA4	30	94	10	94	30	65	60	72	50	72	5
aadB	30	94	10	94	30	68	60	72	50	72	5
aph(3') VI	35	94	3	94	30	49	40	72	60	72	5
adeB	30	95	2	95	60	54	45	72	60	72	10
msr(E)	25	95	5	94	30	68	30	72	45	72	5
mph(E)	25	95	5	94	30	68	30	72	45	72	5
Erm(42)	25	95	5	94	30	68	30	72	45	72	5
parC	30	94	10	94	30	58	60	72	50	72	5
gyrA	30	94	10	94	30	63	60	72	50	72	5
strA	35	94	1	94	30	56	30	72	30	72	2
strB	35	94	1	94	30	56	30	72	30	72	2
sul I	30	94	5	94	15	69	30	72	60	72	7
sul II	30	94	5	94	15	69	30	72	60	72	7
Tet A	25	95	5	94	50	61	30	72	30	72	7
Tet B	25	95	5	94	50	61	30	72	30	72	7
dhfr1	30	94	2	94	60	54	45	72	60	72	10

Supplementary Table 3. Source of isolates.

Source	No. of isolates	Hospital	Isolate number (assigned in this study)
Sputum	16	13 isolates from Arzheen Private Hospital*	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12,
		3 isolates from Nanakali Hospital	16, 17
		2 isolates from Al Jumhury Teaching Hospital	8, 14
Urine	7	3 isolates from Nanakali Hospital	13, 15
		2 isolates from King medical Lab	

Supplementary Table 4. VITEK® 2 Compact system results with *A. baumannii*.

Well no.	Test	Mnemonic	Result	(+) %
2	Ala-Phe-Pro-Arylamidase	APPA	−	0
3	Adonitol	ADO	−	0
4	L-Pyrrolydonyl-Arylamidase	PyrA	−	0
5	L-Arabitol	lARL	−	0
7	D-Cellobiose	dCEL	V	93.8
9	Beta-Galactosidase	BGAL	−	0
10	H2S production	H2S	−	0
11	Beta-N-Acetyl-Glucosaminidase	BNAG	−	0
12	Glutamyl Arylamidace pNA	AGLTp	V	87.5
13	D-Glucose	dGLU	V	93.8
14	Gamma-Glutamyl-Transferase	GGT	−	0
15	Fermentation/ Glucose	OFF	−	0
17	Beta-Glucosidase	BGLU	−	0
18	D-Maltose	dMAL	−	0
19	D-Mannitol	dMAN	−	0
20	D-Mannose	dMNE	V	93.8
21	Beta-Xylosidase	BXYL	−	0
22	Beta−Alanine arylamidace pNA	BAlap	−	0
23	L−Proline Arylamidase	ProA	V	18.8
26	Lipase	LIP	−	0
27	Palatinose	PLE	−	0
29	Tyrocine ARYLAMIDASE	TyrA	V	81.3
31	Urease	URE	V	43.8
32	D-Sorbitol	dSOR	−	0
33	Saccharose/Sucrose	SAC	−	0
34	D-Tagatose	dTAG	−	0
35	D-Trehalose	dTRE	−	0
36	Citrate (Sodium)	CIT	V	87.5
37	Malonate	MNT	V	93.8
39	5-Keto-D-Gluconate	5KG	−	0
40	L−LACTATE Alkalinication	lLATk	V	93.8
41	Alpha-Glucosidase	AGLU	−	0
42	SUCCINATE Alkalinication	SUCT	V	93.8
43	Beta-N-ACETYL-GALACTOSAMINIDASE	NAGA	−	0
44	Alpha-Galactosidase	AGAL	−	0
45	Phosphatase	PHOS	−	0
46	Glycine Arylamidase	GlyA	−	0
47	Ornithine Decarboxylase	ODC	−	0
48	Lysine Decarboxylase	LDC	−	0
53	L−HISTIDINE Accimilation	lHlSa	V	43.8
56	Coumarate	CMT	+	100
57	Beta-Glucoronidase	BGUR	−	0
58	O/129 RESISTANCE (Comp.Vibrio.)	O129R	+	100
59	Glu-Gly-Arg-Arylamidase	GGAA	−	0
61	L−MALATE Accimilation	lMLTa	V	37.5
62	Ellman	ELLM	V	12.5
64	L−LACTATE accimilation	lLATa	V	56.3

+, positive result; -, negative result; V, variable result.

Supplementary Table 5. Biochemical tests of *A. baumannii* isolates.

Strains	Biochemical tests		
	Urease test	**Simmon citrate**	**Kligler iron agar test**
1	Positive	Positive	Alkaline/Alkaline
2	Positive	Positive	Alkaline/Alkaline
3	Positive	Positive	Alkaline/Alkaline
4	Positive	Positive	Alkaline/Alkaline
5	Positive	Positive	Alkaline/Alkaline
6	Positive	Positive	Alkaline/Alkaline
7	Positive	Positive	Alkaline/Alkaline
8	Positive	Positive	Alkaline/Alkaline
9	Positive	Positive	Alkaline/Alkaline
10	Positive	Positive	Alkaline/Alkaline
11	Positive	Positive	Alkaline/Alkaline
12	Positive	Positive	Alkaline/Alkaline
13	Positive	Positive	Alkaline/Alkaline
14	Positive	Positive	Alkaline/Alkaline /H_2S/Gas
15	Positive	Positive	Alkaline/Alkaline
16	Positive	Positive	Alkaline/Alkaline
17	Positive	Positive	Alkaline/Alkaline

Supplementary Table 6. β-lactamase genes detected in each strain.

Isolates No.	β- lactamase gene											
	***bla*$_{OXA-51}$*-like*[1]**		***bla*$_{OXA-23}$*-like*[2]**		***bla*$_{OXA-58}$*-like*[3]**		***bla*$_{TEM}$**		***bla*$_{SHV}$**		***bla*$_{NDM}$**	
	Chr. DNA	Pls. DNA	Chr. DNA	Pls. DNA	Chr. DNA	Pls. DNA	Chr. DNA	Pls. DNA	Chr. DNA	Pls. DNA	Chr. DNA	Pls. DNA
1	-	+	-	+	-	-	+	-	-	-	-	+
2	+	-	+	+	-	-	+	-	-	-	+	-
3	+	-	+	+	-	+	+	-	-	-	-	+
4	+	-	+	+	-	+	+	-	-	-	+	+
5	+	-	+	-	-	+	+	-	-	+	+	-
6	-	-	-	-	-	-	+	-	-	-	-	+
7	+	-	+	-	-	-	+	-	-	-	+	-
8												
9	+	-	-	-	-	-	+	-	-	-	+	+
10	-	+	-	-	-	-	+	-	-	-	+	-
11	+	-	-	-	-	-	+	-	-	+	+	-
12	+	-	-	+	-	-	+	-	-	+	+	+
13	+	-	-	+	-	-	+	-	-	-	+	+
14	-	-	-	+	-	-	+	-	-	-	+	-
15	-	-	-	-	-	-	+	-	-	-	+	-
16	-	-	-	-	-	-	+	-	-	+	+	+
17	-	-	-	-	-	-		-		-	-	-
18	+	+	-		-	-	+	-	-	-	+	-

[1] Includes bla$_{OXA-51}$-, bla$_{OXA-58}$-, bla$_{OXA-64}$-, bla$_{OXA-69}$-, bla$_{OXA-70}$-, bla$_{OXA-71}$-, bla$_{OXA-75}$- and bla$_{OXA-78}$-like genes. [2] Includes bla$_{OXA-23}$-, bla$_{OXA-27}$-, and bla$_{OXA-49}$-like genes.
[3] Includes bla$_{OXA-58}$- and bla$_{OXA-96}$-like genes. Chr. DNA, Chromosome DNA; Pls. DNA, Plasmid DNA.

Supplementary Table 7. Chromosomal resistance genes in *A. baumannii* isolates.

Isolate	parC	gyrA	aadB	aacA4	aph(3) IV	AdeB	sul 1	sul 2	strA	strB	erm	mph	msr	tetA	tetB	dhfr1	No.
1	+	+	-	-	+	-	-	+	+	+	-	-	-	-	+	-	7
2	+	+	-	-	+	+	+	-	+	+	-	-	-	-	+	-	8
3	+	+	-	-	+	+	+	-	+	+	-	-	-	-	+	-	8
4	+	+	-	-	+	+	+	-	+	+	-	-	-	-	+	-	8
5	+	+	-	-	+	+	-	-	-	-	-	-	-	-	+	-	5
6	+	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-	5
7	+	+	-	-	+	+	+	-	+	+	-	-	-	-	+	-	8
8	+	+	-	-	+	+	-	+	+	+	-	-	-	-	+	-	8
9	+	+	-	+	+	-	+	+	-	-	-	-	-	-	-	-	6
10	+	+	-	+	+	+	+	-	+	+	-	-	-	-	+	-	9
11	+	+	-	-	+	+	-	-	+	+	-	-	-	-	+	-	7
12	+	+	-	-	-	+	+	-	+	+	-	-	-	-	+	-	7
13	+	+	-	+	-	+	+	+	+	+	-	-	-	+	+	-	10
14*	+		-	+	-	-	+	-	-	-	-	-		-	+	+	5
15	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	5
16	+	+	-	+	-	+	+	-	+	+	-	-	-	-	-	-	7
17*	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	5
Total %	100	100	0	26.7	73.3	86.7	73.3	40	73.3	73.3	0	0	0	6.7	73.3	0	

*Isolates were identified as not *A. baumannii.* No., total gene number.

Supplementary Table 8. Plasmid resistance genes in *A. baumannii* isolates.

Isolate	parC	gyrA	aadB	aacA4	aph(3)VI	AdeB	sul1	sul2	strA	strB	erm	mph	msr	tet A	tet B	dhfr1	No.
1	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+	-	10
2	+	+	-	+	+	+	+	+	+	+	-	-	+	+	+	+	13
3	+	+	-	+	+	+	+	+	+	+	-	-	-	+	+	-	11
4	+	+	-	+	+	+	+	+	+	+	-	-	+	-	+	+	12
5	+	+	-	-	+	+	+	+	+	+	-	-	-	-	-	-	8
6	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+	-	10
7	+	+	-	-	+	+	+	+	+	+	-	-	+	-	+	+	11
8	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+	-	10
9	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+	-	10
10	+	+	-	+	+	+	+	+	+	+	-	-	+	-	+	+	12
11	+	+	-	+	+	+	+	+	+	+	-	-	-	+	+	+	12
12	+	+	-	+	+	+	+	+	+	+	-	-	+	-	+	-	11
13	+	+	-	+	+	+	+	+	+	+	-	-	-	+	+	-	11
14*	+	+	-	+	+	+	+	+	+	+	-	-	-	+	+	-	11
15	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+	-	10
16	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+	-	10
17*	+	+	-	+	+	+	-		+	+	-	-	-	-	+	-	8
Total %	100	100	0	86.7	100	100	100	100	100	100	0	0	33.3	26.7	93.3	33.3	

*Isolates were identified as not *A. baumannii.* No., total gene number

Supplementary Table 9. Percentage of antibiotics resistance genes detected in *A. baumannii* isolates.

Antibiotic	Genes	% Detection in isolates' chromosome DNA	% Detection in isolates' plasmid DNA
Aminoglycoside	*StrA*	73.3	100
	StrB	73.3	100
	aadB	0	0
	aph(3')-VI	73.3	100
	aacA4	26.7	86.7
Multidrug efflux	*AdeB*	86.7	100
Sulfanamid	*Sul1*	73.3	100
	Sul2	40	100
Tetracyclin	*TetA*	6.7	26.7
	TetB	73.3	93.3
Macrolide	*mph(E)*	0	0
	msr(E)	0	33.3
	erm 42	0	0
Trimethoprime	*Dhfr1*	0	33.3
Fluoroquinolone	*gyrA*	100	100
	parC	100	100

Supplementary Table 10. Source of samples used in this study.

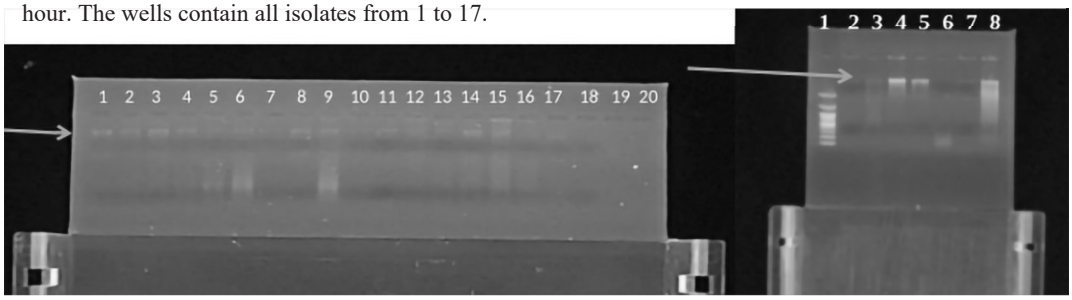
No.	Type of the specimens	Number of the sample (%)	Number of isolates (%)
1	Sputum	93 (16.32%)	16 (17.2%)
2	Urine	355 (62.28%)	7 (1.97%)
3	Blood	43 (7.54%)	0
4	Stool	16 (2.80%)	0
5	Pus	13 (2.28%)	0
6	Swab	38 (6.67%)	0
7	Fluid	12 (2.12%)	0

Supplementary Table 11. Characteristics of SARS-CoV-2 intensive care unit (ICU) patients.

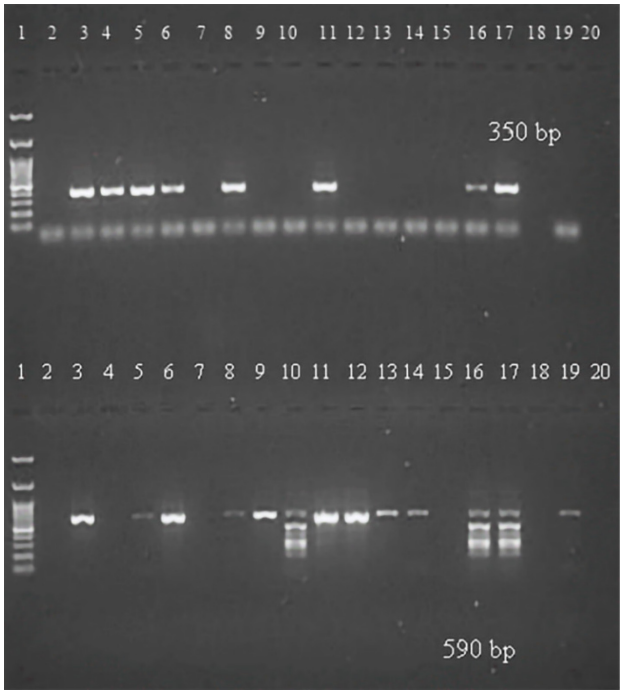
Characteristics	%
Age group	
≤ 50 years old	79%
≥ 50 years old	21%
Sex	
Females	38%
Males	62%
Any diagnosis or problem list entry[1]	
Cardiovascular conditions	21%
Diabetes	55%
Renal failure*	30%
Hypertension	30%
Respiratory conditions	10%
Without any problems	10%

[1] Most ICU cases had multiple comorbidities. *The renal failure was 10% and developed in other disease cases to reach 30%.

Supplementary Figure 1. Plasmid DNA of all isolates on agarose gel 1% electrophoresis at 75 volts for 1 hour. The wells contain all isolates from 1 to 17.

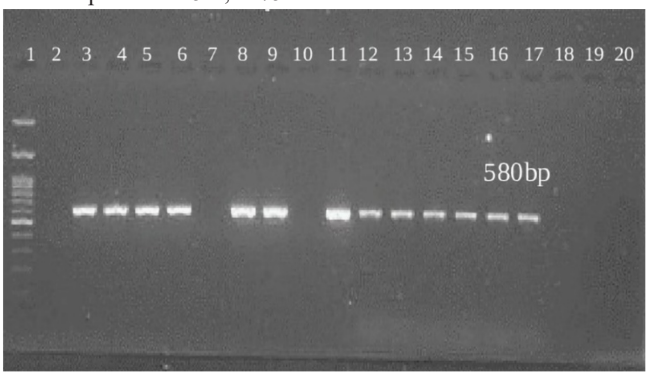


Supplementary Figure 2. Amplification of *A. baumannii* DNA.



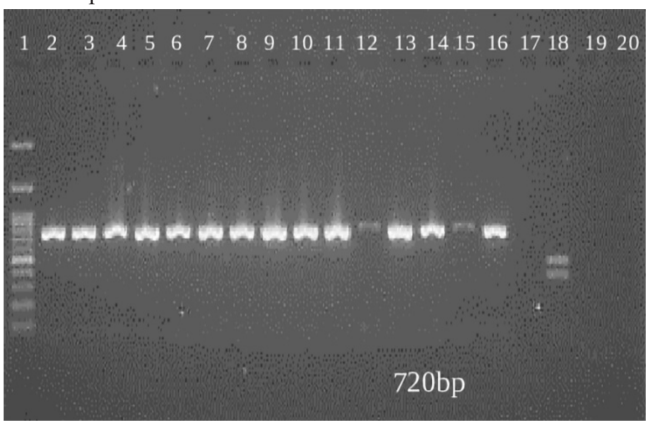
The upper panel shows amplification products using primers OXA SETC and separation by 1.5% agarose gel electrophoresis at 75 volts for 1 hour. Well 1 contains 100 bp DNA ladder; followed by the isolates. The lower panel shows amplification products using primers for blaNDM. Well 1 is 100 bp DNA ladder; followed by isolates no. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17, 14.

Supplementary Figure 3. Amplification of *A. baumannii* with primers OXA SET B and separation by agarose gel electrophoresis 1.5%, at 75 volts for 1 hour.



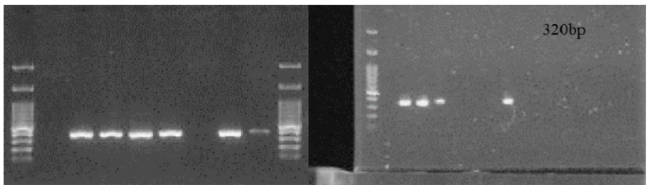
Well 1 contains 100 bp DNA ladder; followed by the isolates.

Supplementary Figure 4. Amplification *of A. baumannii* with primers blaTEM and separation by 1.5% agarose gel electrophoresis at 75 volts for 1 hour.



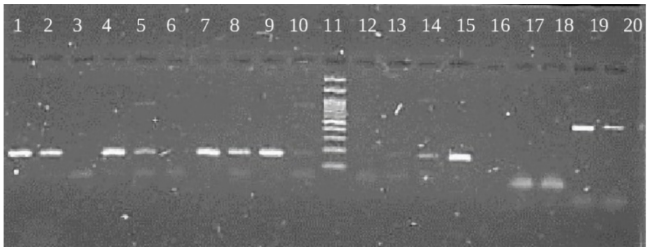
Well 1 is 100 bp DNA ladder; followed by isolates no. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17, 14.

Supplementary Figure 5. Amplification of *A. baumannii* with primers blaOXA-51 and separation by agarose gel electrophoresis 1.5% at 75 volts for 1 hour.



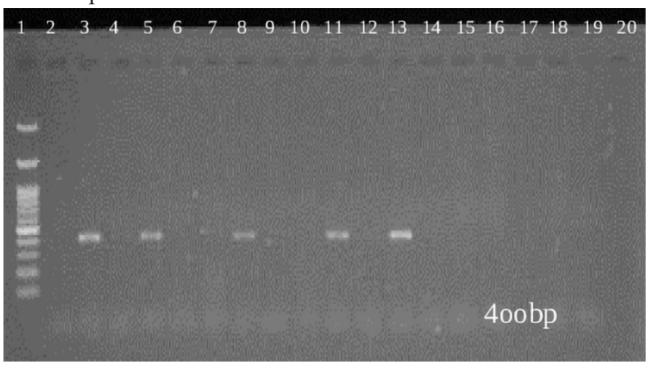
A: well 1 and 10 are 100 bp DNA ladder; followed by isolates no. 1, 2 ,3, 4, 5, 6, 7, 8. B: well 1 is 100 bp DNA ladder; followed by isolates no. 9, 10, 11, 12, 13, 15, 16, 17.

Supplementary Figure 6. Amplification of *A. baumannii* with primers AdeB and separation by agarose gel electrophoresis 1.5% at 75 volts for 1 hour.



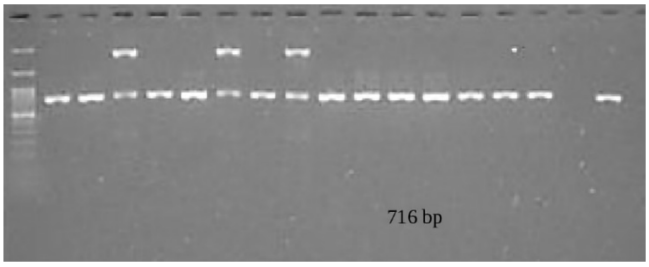
well 11 is 100 bp DNA ladder; well 1: isolate 4, well 2: isolate 5, well 3: isolate 6, well4: isolate 7, well 5: isolate 8, well 6: isolate 9, well 7: isolate 10, well 8: isolate 11, well 9: isolate 12, well10: isolate 13, well 12: isolate14 well 13: isolate 15, well 14: isolate 16, well 15: isolate 17. Wells 19 and 20 are resistance gene aacA4 of isolates 9 and 10.

Supplementary Figure 7. Amplification of *A. baumannii* plasmid with primers msr (E) and separation by agarose gel electrophoresis 1.5% at 75 volts for 1 hour.



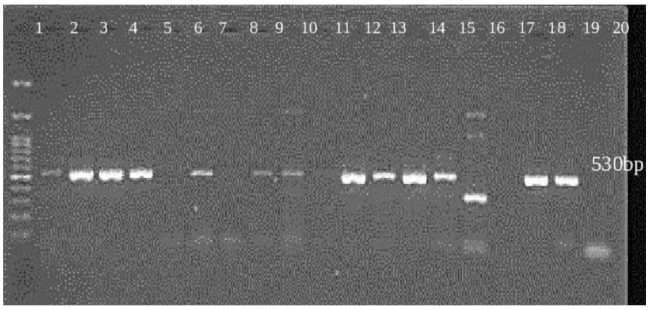
Well 1 is 100 bp DNA ladder; well 2: isolate 1, well 3: isolate 2, well 4: isolate 3, well 5: isolate 4, well 6: isolate 5, well 7: isolate 6, well 8: isolate 9, well 9: isolate 10, well10: isolate 11, well 12: isolate13, well 13: isolate 14, well 14: isolate 15, well 15: isolate 16, well 16: isolate 15, well 17: isolate 16, well 18: isolate 17.

Supplementary Figure 8. Amplification of *A. baumannii* with primers aph (3') and separation by agarose gel electrophoresis 1.5% at 75 volts for 1 hr.



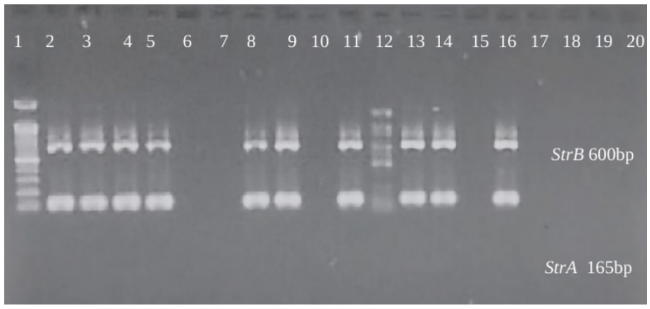
Well 1 is 100 bp DNA ladder, followed by isolates no. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17, -,14, -, -.
-, empty well.

Supplementary Figure 9. Amplification of *A. baumannii* with primers *aacA4* and separation by agarose gel electrophoresis 1.5% at 75 volts for 1 hour.



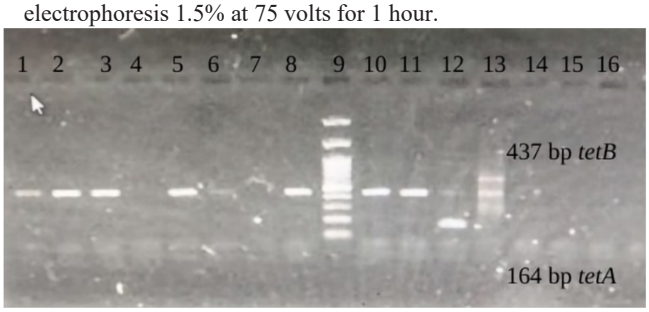
Well 1 is 100 bp DNA ladder; followed by isolates no. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17, -, 15, 17. Well no. 16 is *gyrA* resistance gene and well no. 17 (-) is empty.

Supplementary Figure 10. Amplification of *A. baumannii* with primers *strAB* and separation by agarose gel electrophoresis 1.5% at 75 volts for 1 hour.



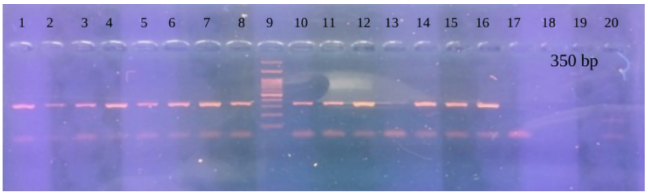
Well 1 is 100 bp DNA ladder; followed by isolates: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 15, and 17.

Supplementary Figure 11. Amplification of *A. baumannii* with primers *tetA* and *tetB* and separation by agarose gel electrophoresis 1.5% at 75 volts for 1 hour.



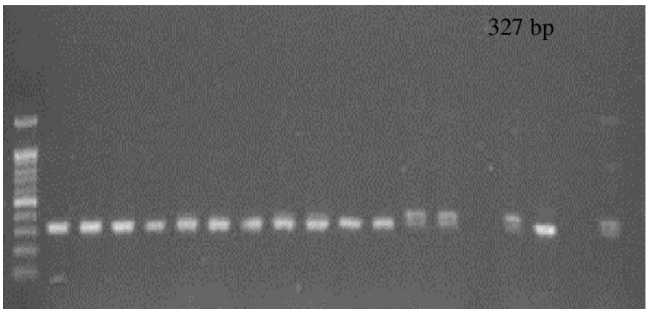
Well 1 is the isolate 1, followed by isolates 2, 3, 6, 7, 8, 9, and 10; well 9 is 100 bp ladder, followed by isolates 11, 12, 13, 14, 16, 15, 17.

Supplementary Figure 12. Amplification of *A. baumannii* with primers *gyrA* and separation by agarose gel electrophoresis 1.5% at 75 volts for 1 hour.



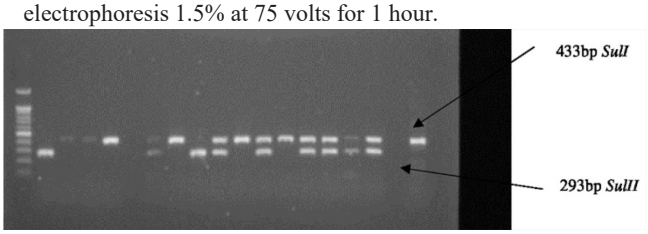
Well 1 is isolate1, followed by isolates: 2, 3, 6, 7, 8, 9, 10; well 9 is the 100bp ladder; followed by isolates 11, 12, 13, 14, 16, 15, 17 and the negative control.

Supplementary Figure 13. Amplification of *A. baumannii* with primers *ParC* and separation by agarose gel electrophoresis 1.5% at 75 volts for 1 hour.



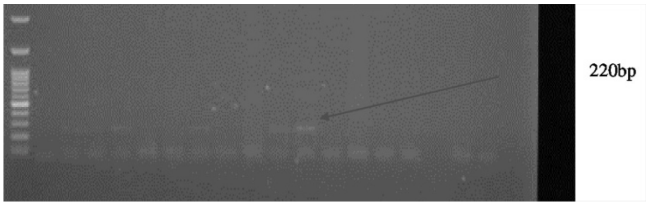
Well 1 is 100 bp DNA ladder; followed by isolates no. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, and 17.

Supplementary Figure 14. Amplification of *A. baumannii* DNA with primers Sul I, Sul II and separation by agarose gel electrophoresis 1.5% at 75 volts for 1 hour.



Well 1 is 100 bp DNA ladder; followed by isolates: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17, empty well, and 14.

Supplementary Figure 15. Amplification of *A. baumannii* plasmid DNA with primers dhfr1 and separation by agarose gel electrophoresis 1.5% at 75 volts for 1 hour.



Well 1 is 100 bp DNA ladder; followed by isolates no. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, empty well, 16, 17.