Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

# Multilocus sequence typing analysis and molecular characterization of carbapenemase related genes in *Acinetobacter baumannii* isolated from hospitalized patients in Erbil city, Iraq

Khelat Adhem Abduljabar[1,2], Ahang Hasan Mawlood[1,3*]

[1] Medical Microbiology Department, College of Health Sciences, Hawler Medical University, Erbil, Iraq
[2] Medical Research Centre, Hawler Medical University, Erbil, Iraq
[3] Department of Medical Laboratory Technique, College of Medical Technology, AL-kitab University, Kirkuk, Iraq

**ARTICLE INFO**

***Original paper***

***Article history:***
*Received: June 25, 2023*
*Accepted: August 01, 2023*
*Published: November 15, 2023*

***Keywords:***

*Acinetobacter baumannii, MLST, oxacillinase gene, metallo β-lactamase gene, clonal complex*

**ABSTRACT**

*Acinetobacter baumannii,* has been recognized by (WHO) as a global priority pathogen. It has been demonstrated to quickly pick up antimicrobial resistance genes. Multilocus sequence typing (MLST) is an unambiguous typing method for identifying accurate and portable nucleotide sequences of internal fragments of multiple housekeeping genes. The present study aimed to determine the sequence type using MLST, genetically define the carbapenem resistance phenotype and clarify the epidemiology of multidrug resistant (MDR) *A. baumannii* in the Kurdistan region of Iraq. Clinical samples were collected from ICU patients. VITEK 2 compact system was used for bacterial identification and antimicrobial susceptibility profile. PCR was used for detecting carbapenemase-related genes. Additionally, MLST was used to evaluate the genetic diversity of carbapenem-resistant isolates using Oxford scheme primers. In this investigation, 63 non-duplicate *A. baumannii* isolates from hospitalized patients were identified. According to CLSI standards, 75% and 73.4% of isolates were resistant to meropenem and imipenem respectively. Tigecycline and colistin were the most effective antibacterial agents. Of the various combinations of carbapenemase genes identified, the most common co-existence of genes present among clinical isolates were $Bla_{OXA-23}$, and $Bla_{OXA-51}$ (95.31%). While the less common combination of genes was detected in 4 isolates (6.25 %) consisting of the co-existence of all tested carbapenemase genes in the present study ($Bla_{OXA-23}$, $Bla_{OXA-51}$, $Bla_{OXA-58}$, $Bla_{IMP}$, $Bla_{VIM}$, $Bla_{NDM}$). Regarding MLST analysis, the results confirmed that ST 556 (n=4) had the greatest frequency rate among clinical isolates, followed by ST 218 (n=3). Notably, the most common worldwide clonal lineage was CC92, which corresponded to (ICII). Only two isolates from ST 441, on the other hand, matched CC109/ ICI. The present investigation revealed a significant level of diversity among *A. baumannii* isolates.

**Doi:** http://dx.doi.org/10.14715/cmb/2023.69.11.18 

## Introduction

Over the past two decades, antimicrobial resistance has become a latent health threat. Our capacity to treat widespread infectious diseases is under threat (1). One such organism, *Acinetobacter baumannii* (*A. baumannii)*, has been recognized by (WHO) as a global priority pathogen and is categorized as an ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*) (2). It has been demonstrated to quickly pick up antimicrobial resistance genes (3). According to (4), it is the primary pathogen responsible for nosocomial infections such as ventilator-associated pneumonia, endocarditis, bacteremia, wound infections, meningitis, and urinary tract infections in immunocompromised patients and in patients with underlying diseases who have spent a long time in the hospital.

The last line of defense for the treatment of severe Gram-negative bacilli infections is carbapenem (meropenem and imipenem). The emergence of inherent or acquired carbapenemases belonging to the class B or class D oxacillinases, as well as decreased cell permeability and mutations that can cause upregulation or downregulation of efflux system activity, may all contribute to the evolution of carbapenem resistance (5). The most comprehensive description of a carbapenem resistance mechanism is prescribed previously (6,7). The hydrolyzing metallo β-lactamases (Class B β-lactamases), including $Bla_{IMP}$, $Bla_{VIM}$, $Bla_{SIM}$, and $Bla_{NDM}$, are typically claimed to be associated with drug resistance mechanisms. Whereas oxacillinases (Class D β-lactamases) are genes encoding OXA β-lactamases are present in plasmids and chromosomes (8). There are now six OXA subclasses that are related to *A. baumannii*: $Bla_{OXA-23}$, $Bla_{OXA-24}$, $Bla_{OXA-51}$, $Bla_{OXA-58}$, $Bla_{OXA-143}$, and $Bla_{OXA-235}$ (7). Also widely reported (9, 10) is the coexistence of class D and class B carbapenem hydrolyzing genes in MDR *A. baumannii* strains. In addition, $Bla_{OXA-51}$ was a key marker for identifying the organism at the species level. The global spread of carbapenem-resistant *A. baumannii* (CRAB), on the other hand, has been related to $Bla_{OXA-23}$, which has been found all over the world (11).

Multi-locus sequence typing (MLST) is an unambi-

* Corresponding author. Email: ahang.mawlood@hmu.edu.krd

guous typing method for identifying accurate and portable nucleotide sequences of internal fragments of multiple housekeeping genes (12). MLST is superior to other typing techniques in a number of ways. MLST is a perfect typing method for an epidemiological investigation since, in addition to resolution, it offers clear typing data to interchange and compare findings between laboratories (13). Additionally, a key advantage of MLST is a derived nomenclature of sequence types (STs), which have been widely and quickly adopted by the community and have enabled the expansion of global collective knowledge on the distribution, spread, and biological characteristics of the main clonal groups (14). The evolution of the *A. baumannii* genomes depends heavily on recombination. This phenomenon is of relevance in several particular locations. According to a number of research, the Oxford method should be adopted in order to take advantage of this behavior for categorization and keep track of the capsular type (14). There are three "worldwide" clonal lineages for *A. baumannii*: international clones (ICs) I, II, and III. The ability of this lineage to integrate novel genes and their adaption to hospital environments can be explained by the ICII's widespread presence in many hospitals throughout the world (8).

The molecular epidemiology of *A. baumannii* infection outbreaks in numerous nations throughout the world has been thoroughly investigated. On the epidemiology of *A. baumannii* and other infections in hospitalized patients in Iraq, however, few studies have been released (15). Additionally, limited molecular typing techniques have been used in earlier research from Iraq, which is crucial for obtaining reliable epidemiological data and understanding the spread of the organism (16-18). To determine the sequence type, genetically define the carbapenem resistance phenotype, and clarify the epidemiology of MDR *A. baumannii* in Kurdistan region of Iraq, the Oxford MLST scheme was used in the current work. Additionally, the genetic characteristics of the antimicrobial resistance profiles contributing to the medications widely used for MDR *A. baumannii* infections in Kurdistan, Iraq, were identified by finding both intrinsic and acquired genetic factors linked with resistance in these isolates.

## Materials and Methods

### Bacterial isolates

Between August 2021 and April 2022, this cross-sectional investigation was conducted in the primary microbiology laboratory at four separate hospitals in the Kurdistan area of Iraq (Emergency Hospital, West Erbil Emergency Hospital, East Erbil Emergency Hospital, and Rizgary Teaching Hospital). A total of 450 clinical samples were collected from ICU patients, including blood, urine, sputum, wound swabs, burns, and Cerebrospinal fluid (CSF). Clinical samples were immediately transported in transport media and cultivated within 30 minutes.

### Culture and identification

The samples were inoculated on blood agar, *Acinetobacter* CHROMagar (CHROMagar, Paris, France), and MacConkey agar medium and incubated at 37°C for 24 hours. Gram staining, culture morphology, and Gram-negative identification (GN) cards in the VITEK 2 compact system (bioMérieux, France) revealed that the culture growth was *A. baumannii.* Each patient's age, gender, date of hospitalization, and culture type were all documented.Throughout the experiment, an ATCC strain of A.baumannii (19606) was donated by Medya Diagnostic Center to serve as a control.

### Antibiotic susceptibility testing

The Vitek 2 Compact system (bioMérieux, Inc., Marcy-l'Etoile, France) was used to test general antimicrobial susceptibilities for *A. baumannii* identification. After overnight incubation at 37°C, the bacterial suspension was prepared for all isolates to make a turbidity adjustment to match the McFarland 0.5 standard in 0.45% sterile sodium chloride solution. The suspensions (2 mL) were put into VITEK 2 ID and AST cards (19). Ceftazidime, imipenem, meropenem, piperacillin, amikacin, tigecycline, tetracyclin, colistin, gentamicin, tazobactam, tobramycin, ciprofloxacin, tri/sulfamethoxazole, and levofloxacin were among the antibiotics utilized. Data were analyzed using Clinical and Laboratory Standards Institute (CLSI) breakpoints, and results were classified as susceptible (S), intermediate (I), or resistant (R) (20).

### DNA extraction

To preserve genetic variety during storage, strains were kept at -70°C in 20% (vol/vol) glycerol in BHI medium and cultivated overnight on MacConkey agar at 37°C. A loopful from a colony was suspended in 500 µl of distilled water. The DNA extraction kit (G-nad and Beta Bayern) was used in line with the manufacturer's instructions. DNA was kept at -20°C until it was needed. The exact primers and PCR conditions for detecting oxacillinases ($Bla_{OXA-51}$, $Bla_{OXA-23}$, $Bla_{OXA-58}$) and metallo B- lactamases ($Bla_{NDM}$, $Bla_{IMP}$, $Bla_{VIM}$) were used as previously published "Table 1". 3 µl MDR *A. baumannii* whole genomic DNA was used as a template for the PCR (5 µl nucleus free water, 10 µl master mix, 1µl per primer). In a thermocycler (Eppendorf, M Germany), a standard PCR amplification program was employed as follows: initial heating to 95°C for 5 minutes, followed by thirty cycles of amplification. Each cycle included three phases: denaturation at 95°C for 30 seconds, annealing at a suitable temperature for 30 seconds, and extension at 72°C for an appropriate time, followed by a final elongation step for 5 minutes at 72°C. Finally, the PCR product was stored at 4°C until they were analyzed. The annealing temperature was selected based on the melting temperature of the screening primer pair (about 5°C lower than the primer melting temperature). The length of the predicted amplified DNA was used to calculate the elongation time. The PCR products were electrophoresized in 1% agarose gel, stained with ethidium bromide, and seen with a UV transilluminator.

### MLST

MLST was used to evaluate the genetic diversity of carbapenem-resistant isolates using Oxford scheme primers listed on the *A. baumannii* MLST database website (https://pubmlst.org/organisms/*acinetobacter-baumannii*). The seven conserved housekeeping genes (*gltA, gryB, gdhB, recA, cpn60, rpoD, and gpi*) were amplified using the methodology given by (23). Magnetic bead purification was used to purify the amplified PCR products. The Applied Biosystems SeqStudio Genetic Analyzer was used for sequencing. The *A. baumannii* MLST database

**Table 1.** Primer sequences, product sizes, annealing temperatures and elongation time for β-lactamase encoding and resistance genes in clinical isolates of *A. baumannii.*

| Targeted genes | Primer sequences (5′-3′) | Product size (bp) | Annealing Temperature (°C) | Elongation time | References |
|---|---|---|---|---|---|
| $Bla_{OXA-51}$ | F:TAATGCTTTGATCGGCCTTG<br>R: TGGATTGCACTTCATCTTGG | 353 bp | 53°C | 30 sec | (6) |
| $Bla_{OXA-23}$ | F: GATCGGATTGGAGAACCAGA<br>R: ATTCTTGACCGCATTTCCAT | 501bp | 53°C | 40 sec | (6) |
| $Bla_{OXA-58}$ | F: AAGTATTGGGGCTTGTGCTG<br>R: CCCCTCTGCGCTCTACATAC | 599bp | 43°C | 40 sec | (6) |
| $Bla_{NDM}$ | F: CACCTCATGTTTGAATTCGCC<br>R: CTCTGTCACATCGAAATCGC | 984bp | 52°C | 60 sec | (21) |
| $Bla_{IMP}$ | F: GGAATAGAGTGGCTTAAYTCTC<br>R: CCAAACYACTASGTTATCT | 188bp | 53°C | 30 sec | (22) |
| $Bla_{VIM}$ | F: GATGGTGTTTGGTCGCATA<br>R: CGAATGCGCAGCACCAG | 390bp | 55°C | 40 sec | (22) |

was used to define the allelic numbers and sequence types (STs). . Kani was given to the isolates name, and a digit number, such as Kani 22, refers to isolate number 22, and so on. Clusters of related STs (designated as clonal complexes; CCs) were evaluated using the Phyloviz 2.0 software's Global Optimal eBURST (goeBURST) (https://www.phyloviz.net/goeburst). The clonal complex was investigated at both the single locus variant (SLV) and double locus variant (DVL) levels to give additional subspecies-level information that could benefit in *A. baumannii* outbreak research, because the majority of outbreaks reported around the world have been proven to contain certain MLST clonal complexes.

## Ethical statement

The research proposal was submitted to the ethics committee of Hawler Medical University's College of Medicine (number: 8-11HMU.ME.EC), and official permission was obtained for sample collection from Rizgare Teaching Hospital, Erbil Emergency Hospital (EMC), West Erbil Emergency Hospital, and East Erbil Emergency Hospital in Erbil city. All patients had agreed to participate in the trial after being assured that their identities would be kept anonymous.

## Statistical analysis

Frequencies and percentages were used to describe the variables in this study. Antimicrobial susceptibility or resistant rates were calculated as the number of susceptible or resistant organism divided by the total number of tested organism, for a given antibiotic.

## Results

### Characteristics of *A. baumannii* isolates:

Sixty three non-duplicate *A. baumannii* isolates were recovered from 450 total clinical samples from hospitalized patients in this study. The patients' ages ranged from 5 months to 62 years, with a mean of 42.3 years. There were 36 males (56.25%) and 27 females (42.85%). Furthermore, the most common source of *A. baumannii* isolation from ICU patients was surgical-site wound infection (36.5%), followed by burn (23.8%), urine (15.87%), sputum (12.69%), blood (7.93%), and CSF (3.17%) "Table 2".

**Table 2.** Age group, Sex and clinical specimen distribution among *A. baumannii.*

| Sex | < 1 | 1-10 | 11-20 | 21-30 | 31-40 | 41-50 | >50 | Total(%) |
|---|---|---|---|---|---|---|---|---|
| Male | - | 1 | 4 | 12 | 6 | 3 | 10 | 36(56.25%) |
| Female | 1 | 1 | 4 | 3 | 3 | 8 | 7 | 27(42.855) |
| Total | 1 | 2 | 8 | 15 | 8 | 12 | 17 | 63(100%) |
| Specimen | | | | | | | | |
| CSF | 1 | - | - | - | 1 | - | - | 2(3.17%) |
| Blood | - | - | 1 | 2 | 1 | - | 1 | 5(7.93%) |
| Sputum | - | - | 1 | 3 | - | 3 | 1 | 8(12.69%) |
| Urine | - | - | 1 | 3 | 1 | 1 | 4 | 10(15.87%) |
| Burn | - | 2 | 1 | 1 | 2 | 5 | 4 | 15(23.80%) |
| Wound | - | - | 4 | 6 | 4 | 2 | 7 | 23(36.50%) |
| Total | 1 | 2 | 8 | 15 | 9 | 10 | 17 | 63(100%) |

**Table 3.** Antimicrobial susceptibility profile among *A. baumanii* isolates.

| Antibiotics | Susceptible | | Intermediate | | Resistant | |
|---|---|---|---|---|---|---|
| | Number | % | Number | % | Number | % |
| Meropenem | 11 | 17.2 | 5 | 7.8 | 48 | 75 |
| Imipenem | 12 | 18.8 | 5 | 7.8 | 47 | 73.4 |
| Ceftazidime | 7 | 10.9 | 0 | 0 | 57 | 89.1 |
| Tazobactam | 4 | 6.3 | 2 | 3.1 | 58 | 90.6 |
| Piperacillin | 0 | 0 | 2 | 3.1 | 62 | 96.9 |
| Tigecycline | 47 | 73.4 | 17 | 26.6 | 0 | 0 |
| Levofloxacin | 11 | 17.2 | 1 | 1.6 | 52 | 81.3 |
| Ciprofloxacin | 9 | 14.1 | 15 | 23.4 | 40 | 62.5 |
| Gentamycin | 5 | 7.8 | 6 | 9.4 | 53 | 82.8 |
| Tobramycin | 13 | 20.3 | 6 | 9.4 | 45 | 70.3 |
| Amikacin | 8 | 12.5 | 5 | 7.8 | 51 | 79.7 |
| Tetracycline | 12 | 18.8 | 32 | 50 | 20 | 31.3 |
| Colistin | 61 | 95.3 | 3 | 4.7 | 0 | 0 |
| Tri/sulfamethoxazole | 4 | 6.2 | 3 | 4.7 | 57 | 89.1 |

**Antimicrobial resistance pattern of MDR *A. baumannii* clinical isolates**

"Table 3" shows the resistance rate of *A. baumannii* isolates to a range of antibiotics. *A. baumannii* isolates demonstrated high resistance to piperacillin (96.9%), tazobactam (90.6%), ceftazidime and tri/sulfamethoxazole (both 89.1%), gentamycin ( 82.8%), and levofloxacin (81.3%). The susceptibility of *A. baumannii* to colistin and Tigecycline was highest (95.3%) and (73.4%) respectively.

**Distribution of carbapenem-resistant genes in *A. baumannii* isolates**

As shown in "Table 4" PCR screening analysis was utilized to identify genes responsible for metallo β-lactamase (MBL) and class D carbapenem hydrolyzing enzyme in *A. baumannii* isolates. All *A. baumannii* isolates tested positive for at least one carbapenemase resistance gene. The intrinsic β-lactamase $Bla_{OXA-51}$ gene was found in 63 CRAB (98.4%). Other class D β-lactamase genes, such as $Bla_{OXA-23}$ and $Bla_{OXA-58}$, were found in 62 (96.8%) and 36 (56.3%) of CRAB isolates, respectively. Among the MBL genes, It was discovered that (45, 70.3% and 43, 67.2%) were positive for $Bla_{IMP}$ and $Bla_{VIM}$ genes, respectively. Only 18 (28.1%) of the isolates carried $Bla_{NDM}$.

**Distribution of carbapenem-resistant gene combination among *A. baumannii*.**

Of the various combinations identified, the most common co-existence of genes present among clinical isolates were $Bla_{OXA-23}$, $Bla_{OXA-51}$ (95.31%) "Table 5", followed

**Table 4.** Distribution of Carbapenem resistance genes among *A. baumanii* isolates.

| Genes | Number | % |
|---|---|---|
| $Bla_{OXA-51}$ | 63 | 98.4 |
| $Bla_{OXA-23}$ | 62 | 96.9 |
| $Bla_{OXA-58}$ | 36 | 56.3 |
| $Bla_{IMP}$ | 45 | 70.3 |
| $Bla_{VIM}$ | 43 | 67.2 |
| $Bla_{NDM}$ | 18 | 28.1 |

**Table 5.** Distribution of carbapenem-resistant genes combination among *A. baumanii* isolates.

| Gene combination | Number | % |
|---|---|---|
| $Bla_{OXA-23}$, $Bla_{OXA-51}$ | 61 | 95.3 |
| $Bla_{OXA-23}$, $Bla_{OXA-51}$, $Bla_{OXA-58}$ | 32 | 50 |
| $Bla_{OXA-23}$, $Bla_{OXA-51}$, $Bla_{OXA-58}$, $Bla_{IMP}$ | 24 | 37.5 |
| $Bla_{OXA-23}$, $Bla_{OXA-51}$, $Bla_{OXA-58}$, $Bla_{IMP}$, $Bla_{VIM}$ | 19 | 29.7 |
| $Bla_{OXA-23}$, $Bla_{OXA-51}$, $Bla_{OXA-58}$, $Bla_{IMP}$, $Bla_{VIM}$, $Bla_{NDM}$ | 4 | 6.25 |
| $Bla_{OXA-51}$, $Bla_{OXA-58}$ | 34 | 53.1 |
| $Bla_{OXA-51}$, $Bla_{OXA-58}$, $Bla_{IMP}$ | 26 | 40.6 |
| $Bla_{OXA-51}$, $Bla_{OXA-58}$, $Bla_{IMP}$, $Bla_{VIM}$ | 20 | 31.3 |
| $Bla_{OXA-51}$, $Bla_{OXA-58}$, $Bla_{IMP}$, $Bla_{VIM}$, $Bla_{NDM}$ | 5 | 7.8 |
| $Bla_{OXA-58}$, $Bla_{IMP}$ | 25 | 39.1 |
| $Bla_{OXA-58}$, $Bla_{IMP}$, $Bla_{VIM}$ | 19 | 29.7 |
| $Bla_{OXA-58}$, $Bla_{IMP}$, $Bla_{VIM}$, $Bla_{NDM}$ | 5 | 7.8 |
| $Bla_{IMP}$, $Bla_{VIM}$ | 29 | 45.3 |
| $Bla_{IMP}$, $Bla_{VIM}$, $Bla_{NDM}$ | 9 | 14.1 |
| $Bla_{VIM}$, $Bla_{NDM}$ | 13 | 20.3 |

by co-existence of ($Bla_{OXA-51}$, $Bla_{OXA-58}$) and ($Bla_{OXA-23}$, $Bla_{OXA-51}$, $Bla_{OXA-58}$) which was detected in 53.12 % and 50% respectively. While the less common combination of genes was detected in 4 isolates (6.25 %) consisting of the co-existence of all tested carbapenemase genes in the present study ($Bla_{OXA-23}$, $Bla_{OXA-51}$, $Bla_{OXA-58}$, $Bla_{IMP}$, $Bla_{VIM}$, $Bla_{NDM}$).

### MLST and distribution of sequence types (STs) among isolated *A. baumannii*

In this study, the amplification of seven housekeeping genes was performed and the PCR result was shown in "Figure 1".One of the forty-two clinical *A. baumannii* isolates tested by MLST was the reference strain Kani16, which was related to ST 195. The remaining 41 isolates were classified into 33 different sequence types (STs). Among the 33 STs found, 21/33 (63.6%) STs were newly assigned, accounting for 21/42 (50%) of total isolates. Only one isolate (Kani 28) was untypeable due to the inability of one of the alleles *gpi* to be amplified "Table 6".

The results confirmed that ST 556 (n=4) had the greatest frequency rate among clinical isolates, followed by ST 218 (n=3). Notably, ST 195, 387, and 441 were all present in an equal number of *A. baumannii* isolates (n=2). Furthermore, the remaining STs were found to be duplicated only once among *A. baumannii* isolates "Table 6". Remarkably, the most common worldwide clonal lineage is CC92, which corresponded to ICII. Only two isolates from ST 441, on the other hand, matched CC109/ ICI. The present investigation revealed a significant level of diversity among *A. baumannii* isolates.

To detect the clonal complex distribution among studied isolates, an online geoBurst analysis tool (http://www.phyloviz.net/goeburst/#Description) was used. The result was shown that thirty-two separate STs were grouped into 5 clonal complexes (CCs) and 9 singletons. STs (218-2882-2879-195), (502-2872), (556-2885), (350-556), (2589-1624) in CC1, STs (122-441-2884) in CC2, STs (2883-2841-2881) in CC3 were grouped at level of single-locus variants (SLV); among them 9 STs were allocated for the first time. STs (195-2882) in CC1, STs (441-2876) in CC2, STs (2874-1641) in CC4, and STs (2870-2873) in CC5 have been pooled at level double-locus variants (DLV), with 5 newly assigned STs "Figure 2".

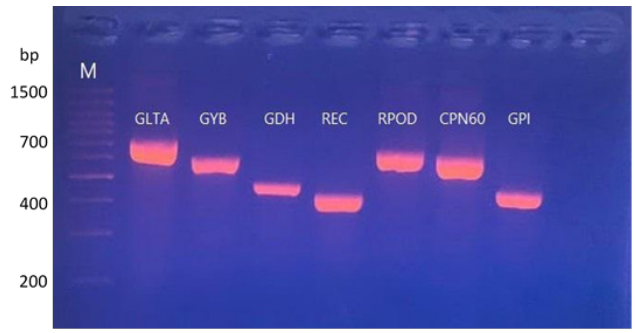


**Figure 1. Agarose gel electrophoresis of PCR amplification of 7-housekeeping genes**; **MLST genes**. Lane 1: marker 100 bp, Lane 2: *gltA* (722 bp); Lane 3: *gyrB* (594 bp); Lane 4: *gdhB* (420 bp); Lane 5: *recA* (425 bp); Lane 6: *rpoD* (672 bp); Lane 7: *cpn60* (640 bp); Lane 8: *gpi* (456 bp); Lane 9: negative control.

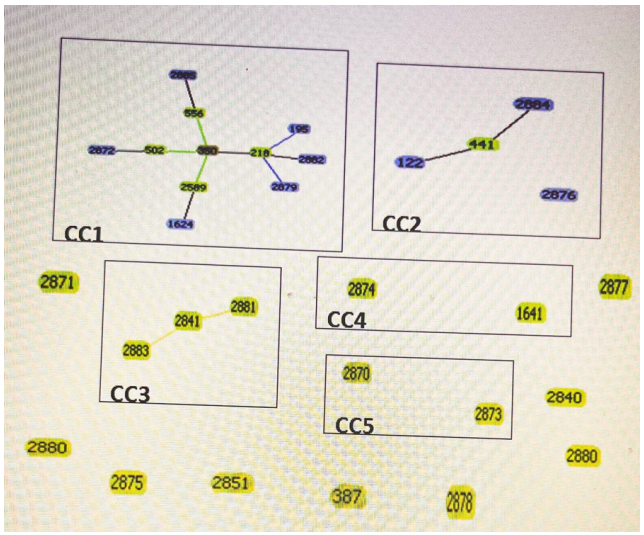


**Figure 2.** shows an eBURST analysis of all *A. baumannii* isolates in our investigation using MLST data. STs are represented by numbers. A line connects STs in the same cluster designated as clonal complexes; CCs. The other nine (9) STs in the figure were unrelated to any group and were singletons.

## Discussion

Carbapenem is a significant therapeutic for dangerous hospital-acquired infections as well as for the treatment of patients infected with multidrug-resistant organisms, notably *A. baumannii* (24). This could be attributed to their broader antibacterial action and low side effects (5). WHO has designated CRAB as one of the most difficult pathogens to control and treat (25).

The prevalence rate of CRAB varies from one country to another (26). The current investigation found that the majority of clinical isolates (47/64, 73.4%) were resistant to both meropenem and imipenem, as well as significant resistance to piperacillin, tazobactam, ceftazidime, tri/sulfamethoxazole, gentamycin, and levofloxacin. Noteworthy, there were still a few antimicrobial medicines that were effective against the majority of CRAB, such as tigecycline (47/64) 73% and colistin (61/64) 95%, respectively. This is comparable to a recent study conducted in Bagdad city (17), who discovered that the drug resistance rate of *A. baumannii* to imipenem and meropenem was greater than 80%, whereas all isolates were sensitive to colistin and tigecycline. This investigation found that 62% and 89% of isolates were resistant to ciprofloxacin and tri/sulfamethoxazole, respectively, Radhi and Al-Charrakh conduct a study in Babylon province, Iraq and discovered a lower rate of resistance to the same antibiotics, which was 20% and 60%, respectively (18). Furthermore, (27) conducted a similar study in Tehran and discovered that all isolates of *A. baumannii* were resistant to the majority of drugs. CRAB isolates limits treatment options, potentially leading to greater morbidity and mortality rates (28). Overall, studies have demonstrated that MDR *A. baumannii* strains can acquire antimicrobial resistance through insertion sequences (IS), integrons, and resistance islands (RIs). Because of its particular property, *A. baumannii* is a problematic nosocomial infection and one of the most dangerous threats to hospitalized patients (24).

Several mechanisms might lead to carbapenem resistance in *A. baumannii*; however, the most common mechanism reported is the presence of oxacillinases and metallo-lactamase (29). As a result, screening for carbapenemase

**Table 6.** Description of the STs (novel and previously recorded) found in the current study based on the Oxford scheme (*A. baumannii*).

| Id | Isolate name | Source | Sex | Age | Oxford MLST allele scheme | | | | | | | ST |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *gltA* | *gyrB* | *gdhB* | *recA* | *cpn60* | *gpi* | *rpoD* | |
| 8719 | Kani 2 | Wound | Male | 55 | 33 | 31 | 3 | 28 | 1 | 107 | 5 | 2840** |
| 8720 | Kani20 | Wound | Female | 16 | 1 | 1 | 66 | 60 | 33 | 269 | 41 | 2841** |
| 8726 | Kani14 | Wound | Male | 25 | 1 | 12 | 3 | 2 | 2 | 102 | 3 | 350 |
| 8727 | Kani15 | Wound | Female | 3 | 1 | 3 | 3 | 2 | 2 | 96 | 3 | 195 |
| 8728 | Kani16 | Blood | Reference | - | 1 | 3 | 3 | 2 | 2 | 96 | 3 | 195 |
| 8729 | Kani1 | CSF | Female | 5M | 1 | 12 | 3 | 2 | 2 | 94 | 3 | 2589 |
| 8730 | Kani8 | Blood | Male | 29 | 1 | 50 | 3 | 2 | 2 | 94 | 3 | 1624 |
| 8731 | Kani10 | Wound | Male | 54 | 1 | 3 | 3 | 2 | 2 | 102 | 3 | 218 |
| 8732 | Kani18 | Wound | Male | 56 | 1 | 15 | 4 | 11 | 4 | 140 | 4 | 387 |
| 8733 | Kani19 | Wound | Female | 23 | 1 | 15 | 4 | 11 | 4 | 140 | 4 | 387 |
| 8734 | Kani21 | Wound | Male | 57 | 1 | 15 | 2 | 28 | 1 | 157 | 32 | 1641 |
| 8744 | Kani25 | Wound | Female | 45 | 1 | 305* | 167 | 67 | 2 | 289 | 89 | 2851** |
| 8940 | Kani22 | Wound | Male | 19 | 129 | 305 * | 167 | 67 | 124 | 102 | 89 | 2870** |
| 8941 | Kani 28 | Wound | Female | 43 | 1 | 96 | 3 | 29 | 2 | - | 3 | ND |
| 8942 | Kani 29 | Wound | Male | 39 | 1 | 96 | 3 | 2 | 2 | 100 | 3 | 2872** |
| 8943 | Kani 6 | Wound | Male | 24 | 10 | 12 | 4 | 11 | 4 | 100 | 5 | 441 |
| 8944 | Kani 41 | Blood | Male | 16 | 1 | 3 | 3 | 2 | 2 | 102 | 3 | 218 |
| 8945 | Kani 30 | Wound | Male | 44 | 129 | 305 * | 59 | 67 | 124 | 100 | 89 | 2873** |
| 8946 | Kani 33 | CSF | Male | 36 | 18 | 12 | 3 | 2 | 2 | 144 | 3 | 2885** |
| 8947 | Kani 35 | Urine | Male | 25 | 1 | 12 | 2 | 28 | 4 | 157 | 32 | 2874** |
| 8948 | Kani 38 | Urine | Female | 23 | 18 | 45 | 3 | 60 | 4 | 269 | 79 | 2886** |
| 8949 | Kani 36 | Wound | Male | 19 | 1 | 15 | 13 | 60 | 30 | 476 * | 2 | 2875** |
| 8950 | Kani 40 | Blood | Female | 42 | 18 | 12 | 148 | 11 | 4 | 100 | 5 | 2876** |
| 8951 | Kani 48 | Wound | Male | 35 | 13 | 90 | 3 | 6 | 101 | 80 | 5 | 2877** |
| 8952 | Kani 57 | Urine | Female | 55 | 129 | 246 | 177 | 7 | 211 * | 80 | 258 * | 2878** |
| 8953 | Kani 39 | Wound | Female | 27 | 10 | 12 | 4 | 11 | 4 | 100 | 5 | 441 |
| 8954 | Kani 44 | Wound | Male | 31 | 1 | 3 | 3 | 2 | 2 | 102 | 3 | 218 |
| 8955 | Kani 3 | Wound | Female | 42 | 1 | 3 | 3 | 2 | 2 | 98 | 3 | 2879 ** |
| 8956 | Kani 32 | Wound | Female | 53 | 18 | 96 | 3 | 6 | 4 | 100 | 5 | 2880** |
| 8957 | Kani 34 | Wound | Male | 51 | 1 | 1 | 42 | 60 | 33 | 269 | 41 | 2881** |
| 8958 | Kani 42 | CSF | Male | 61 | 1 | 12 | 3 | 2 | 2 | 100 | 3 | 502 |
| 8959 | Kani 52 | Wound | Male | 28 | 1 | 12 | 3 | 2 | 2 | 144 | 3 | 556 |
| 8960 | Kani 53 | Wound | Male | 23 | 1 | 3 | 3 | 29 | 2 | 102 | 3 | 2882** |
| 8961 | Kani 55 | Wound | Female | 37 | 1 | 1 | 3 | 60 | 33 | 269 | 41 | 2883** |
| 8962 | Kani 59 | Sputum | Male | 60 | 10 | 12 | 42 | 11 | 4 | 100 | 5 | 2884** |
| 8963 | Kani 60 | Sputum | Male | 25 | 1 | 12 | 3 | 2 | 2 | 144 | 3 | 556 |
| 8964 | Kani 61 | Sputum | Female | 42 | 10 | 12 | 4 | 6 | 4 | 100 | 5 | 122 |
| 8965 | Kani 64 | Wound | Male | 41 | 1 | 12 | 3 | 2 | 2 | 144 | 3 | 556 |
| 8966 | Kani 13 | Wound | Female | 33 | 1 | 3 | 3 | 6 | 92 | 100 | 5 | 2871** |
| 9323 | Kani 62 | Urine | Female | 52 | 1 | 12 | 3 | 2 | 2 | 144 | 3 | 556 |
| 9324 | Knai 63 | Wound | Male | 33 | 10 | 12 | 3 | 6 | 92 | 100 | 5 | 2894** |

resistance genes is essential in order to decide on the best potential therapy regimen. The distribution of the examined genes encoding oxacillinases ($Bla_{OXA-51}$, $Bla_{OXA-23}$, $Bla_{OXA-58}$) and metallo-lactamases ($Bla_{IMP}$, $Bla_{VIM}$, and $Bla_{NDM}$) is shown in "Table 4".The current investigation found that 98% of the total isolates tested positive for *A. baumannii* ($Bla_{OXA-51}$ ). $Bla_{OXA-23}$ was detected in almost 96% of confirmed isolates, and $Bla_{OXA-58}$ was detected in 56% of the isolates. These findings are comparable to those of Ganjo et al, who found that $Bla_{OXA-51}$ and $Bla_{OXA-23}$ were more abundant in A. baumannii isolates, but in contrast to our findings, $Bla_{OXA-58}$ was not found in any isolate (30). In line with this finding, (31) identified a significant prevalence of $Bla_{OXA-23}$ bearing *A. baumannii* strains in Iraqi

patients. In Asia, the acquired $Bla_{OXA-23}$ is the primary genetic determinant. Through conjugation, the $Bla_{OXA-23}$ gene on a plasmid can be transferred between *A. baumannii*. As a result, antibiotic-resistant bacteria are spreading fast over the world (32). Furthermore, in contrast to the current data, (29) reported in Erbil city that the $Bla_{OXA-58}$ gene was not identified in any of the *A. baumannii* isolates. Another study published in Egypt found that all isolates contained $Bla_{OXA-51}$, but a lesser percentage of isolates (78% vs. 96% in our study) tested positive for $Bla_{OXA-23}$. The presence of $Bla_{OXA-51}$ was discovered to have minimal carbapenemase activity; however, when this gene is overexpressed, it exhibits considerable carbapenemase activity. As a result, resistance to carbapenem in *A. baumannii* required the existence of other acquired oxacillinases such as $Bla_{OXA-23,}$ $Bla_{OXA-143,}$ $Bla_{OXA-24,}$ and $Bla_{OXA-143}$ enzyme (4). Furthermore, (6) utilized a PCR screening test for a total of 196 clinical isolates and found that 48.4% were $Bla_{OXA-51,}$ 46.3% were $Bla_{OXA-23,}$ and 5.3% were $Bla_{OXA-58,}$ which was lower than the results obtained in this investigation.

In addition to oxacillinases, the distribution of MBL genes was screened in the current study, which can be called the second most prevalent carbapenemases in CRAB. It has been found that the percentage of the $Bla_{IMP,}$ $Bla_{VIM,}$ and$Bla_{NDM}$ was 70%, 67%, and 26%, respectively. This rate was higher than the results conducted by (31) in Sulaimani city, Iraq, where 10.7% and 2.8% of CRAB isolates contained $Bla_{IMP}$ and $Bla_{VIM}$, respectively; however, $Bla_{NDM}$was not discovered in any *A. baumannii* isolate. In a study on the epidemiology of common resistant bacterial infections in Arab League nations, (26) discovered that the prevalence of CRAB was highest in Iraq, followed by Lebanon and Syria (89%, 70.5%, and 64.0%, respectively) in the Levant. The high resistance to this class of antibiotics may be attributable to their widespread use in Iraqi hospitals (18). Furthermore, variants in the distribution of different carbapenamase genes in different nations may be associated with varied ecological situations, antibiotic therapy programs, and variant antibiotype patterns (3).

The co-existence of a number of intrinsic and acquired carbapenemase-encoding genes has been observed, with acquired carbapenemase-encoding genes being more prevalent. Isolates with multiple acquired carbapenemase-encoding genes account for 61/64 (95.3%) of all CRAB isolates. Many investigations in the Mediterranean region (8) and China (33, 34) have found clones bearing numerous carbapenemase-encoding genes. It appears that the rising combination of these genes may result in an increase in carbapenem resistance among *A. baumannii* isolates (35).

A notable finding in this investigation was the co-existence of oxacillinase and MBL in some isolates, which has been observed in numerous studies conducted in countries with uncontrolled antibiotic usage (8). The connection of these genes by class 1 (occasionally class 3) integrons, which are embedded in transposons, could explain the increased occurrence of co-existing MBL. Integrons allow resistance genes to flow between integrons in plasmids, and plasmids allow genetic material to be transferred to different bacteria. Our findings verified the presence of $Bla_{oxa-23}$, $Bla_{oxa-51}$, $Bla_{oxa-58}$, $Bla_{IMP}$, $Bla_{VIM}$, and $Bla_{NDM}$ in 4 (6.3%) of the cases. In line with this finding, (32) discovered in Nipal a greater incidence of isolates 6 (13.6%) harboring the $Bla_{NDM}$ gene and $Bla_{oxa-51}$ in addition to $Bla_{oxa-23}$. In contrast to the current work, they did not find MBL genes such as $Bla_{VIM}$ and $Bla_{IMP}$.

MLST typing, which is frequently utilized in the characterisation of genotypes circulating in hospitals (36), was employed to analyze the *A. baumannii* population structure.We detected 32 *A. baumannii* STs in our study, 21 of which were previously unknown genotypes. In fifteen isolates, new alleles were found in novel allelic combinations. The majority of them were SLVs and DLVs from existing clones. STs 2879 and 2882, for example, were SLVs and DLVs of ST 195. Furthermore, ST2884 and ST2876 were the SLV and DLV of the ST 441. These data suggest that the novel STs were produced by local genetic alterations in existing clones. It also shows that highly adapted *A. baumannii* genotypes from CC92 have diffused and grown locally, culminating in the creation of different SLVs or DLVs in CC92.

Five new STs, on the other hand, had novel alleles that resulted in a new allelic profile and have been assigned to the MLST website (ST 2851, ST 2870, ST 2873, 2875, and 2878). Some of the STs found belonged to clonal complexes, with 16 isolates belonging to the CC92/CC2 (Oxford/Pasteur) scheme, which corresponds to IC2, and just four isolates belonging to the CC109/CC1 (Oxford/Pasteur) scheme, which corresponds to IC1. The majority of reported outbreaks worldwide have been shown to contain MLST IC2 and IC1 (37). According to the current study, the carbapenemase expressing genes $Bla_{oxa-23}$ was discovered in the majority of these isolates. According to prior findings, the majority of clinical isolates from Asia, the United States, and Australia belonged to CC92 and had $Bla_{oxa-23}$. The extensive circulation of imipenem-resistant, BlaOXA-23 generating CC92 is unmistakably the reason for China's fast-rising carbapenem resistance (38). Because novel antibiotics to treat CRAB are unavailable, and there is uncertainty about whether new treatments would ever be useful, infection control policy and practice based on the framework of these phylogenetic and epidemiological findings is crucial in slowing the spread of CRAB. Without such steps, we will enter a period in which CRAB infections and minor injuries will be fatal (1).

In conclusion, The current investigation discovered substantial levels of carbapenem resistance in *A. baumannii*. $Bla_{oxa-23}$ and $Bla_{IMP}$ have been identified as important carbapenem resistance factors in CRAB isolates. Additionally, 33 different STs were found, 21 of which are unique. The isolates are related to international spread clones with extraordinarily high resistance rates, suggesting that *A. baumannii* can survive in hospitals by gaining several resistance genes. In light of similar findings from Iraq, doctors and healthcare workers must be aware of the *A. baumannii* population in order to implement effective infection control procedures and antimicrobial treatments. Thus, molecular and genomic technologies must be used to monitor the prevalence of *A. baumannii* clinical isolates and may aid in the implementation of appropriate interventions, contributing to hospital infection control.

**Interest conflict**

The authors declare that they do not have any conflict of interest in the research work and publish the article.

**Author's contribution**

Ahang Hasan Mawlood created the plan concept, was in charge of interpreting the results, oversaw the entire pro-

ject, and revised the final paper. Khalat Adham Abduljabar carried out the experiments and prepared the paper. Both authors read the article, agreed on the final manuscript, and were approved to be responsible for all aspects of the work.

## References


1. Hamidian M, Nigro SJ. Emergence, molecular mechanisms and global spread of carbapenem-resistant *Acinetobacter baumannii*. Microb Genom 2019;5(10):e000306. doi: 10.1099/mgen.0.000306.
2. Panda SK, Buroni S, Swain SS, Bonacorsi A, da Fonseca Amorim EA, Kulshrestha M, da Silva LC, Tiwari V. Recent Advances to Combat ESKAPE Pathogens with Special Reference to Essential Oils. Front Microbiol 2022;13:1029098. doi: org/10.3389/fmicb.2022.1029098
3. Azimi L, Talebi M, Pourshafie MR, Owlia P, Lari AR. Characterization of carbapenemases in extensively drug resistance *Acinetobacter baumannii* in a burn care center in Iran Int J Mol Cell Med 2015; 4: 46–53.
4. Abd El-Baky RM, Farhan SM, Ibrahim RA, Mahran KM, Hetta HF. Antimicrobial resistance pattern and molecular epidemiology of ESBL and MBL producing *Acinetobacter baumannii* isolated from hospitals in Minia Egypt. Alex J Med 2020 ;56(1): 3-14. doi: 10.1080/20905068.2019.1707350.
5. Olu-Taiwo MA, Opintan JA, Codjoe FS. Metallo-beta-lactamase-producing Acinetobacter spp. from clinical isolates at a tertiary carehospital in Ghana. Biomed Res Int 2020;2020:3852419. doi:101125/2020/3852419.
6. Hou C, Yang F. Drug-resistant gene of blaOXA-23, blaOXA-24, blaOXA-51 and blaOXA-58 in *Acinetobacter baumannii* Int J Clin Exp Med 2015;8(8):13859–13863. ISSN:1940-5901/IJCEM0008809.
7. Beigverdi R, Sattari-Maraji A, Emaneini M, Jabalameli F. Status of carbapenem-resistant *Acinetobacter baumannii* harboring carbapenemase: First systematic review and meta-analysis from Iran. Infect Genet Evol 2019; 73: 433–443. doi.org/10.1016/j.meegid.2019.06.008.
8. Wasfi R, Rasslan F, Hassan SS, Ashour HM, Abd El-Rahman OA. Co-existence of carbapenemase-encoding genes in *Acinetobacter baumannii* from cancer patients. Infect Dis Ther 2021;10:291-305.doi:10.1007/s40121-020-00369-4.
9. Ranjbar R, Farahani A. Study of genetic diversity, biofilm formation, and detection of Carbapenemase, MBL, ESBL, and tetracycline resistance genes in multidrug-resistant *Acinetobacter baumannii* isolated from burn wound infections in Iran. Antimicrob Resist Infect Control 2019; 8(1). doi: 10.1186/s13756-019-0612-5.
10. Svetlana AK, Korobova AG, Anastasiya VF, Frolova IN, Savochkina YA, Galina AK. Detection of acquired carbapenemase genes among* *Acinetobacter baumannii** isolated from blood culture in patients with hematological malignancies.CMAC 2019; 1;21(1):56-60. doi: 10.36488.
11. Makke G, Bitar I, Salloum T, Panossian B, Alousi S, Arabaghian H. Whole-genome-sequence-based characterization of extensively drug-resistant *Acinetobacter baumannii* hospital outbreak. MSphere 2020; 5(1): pp.e00934-19. doi: 10.1128/msphere.00934-19.
12. Zhong Q, Xu W, Wu Y, Xu H. Clonal spread of carbapenem nonsusceptible *Acinetobacter baumannii* in an intensive care unit in a teaching hospital in China. Ann. Lab. Med. 2012; 1, 32(6):413-9. doi: https://doi.org/10.3343/alm.2012.32.6.413.
13. Kumar S, Patil PP, Singhal L, Ray P, Patil PB and Gautam V. Molecular epidemiology of carbapenem-resistant *Acinetobacter baumannii* isolates reveals the emergence of blaOXA-23 and blaNDM-1 encoding international clones in India Infect Genet Evol 2019; 75, p.103986. doi: doi.org/10.1016/j.meegid.2019.103986.
14. Gaiarsa S, Batisti Biffignandi G, Esposito EP, Castelli M, Jolley KA, Brisse S, Sassera D, Zarrilli R. Comparative analysis of the two *Acinetobacter baumannii* multilocus sequence typing (MLST) schemes. Front Microbiol 2019; 3,10:930 doi: doi.org/10.3389/fmicb.2019.0 0930.
15. Kadhom H, Ali M. Epidemiological molecular analysis of *Acinetobacter baumannii* isolates using a multilocus sequencing typing and global lineage. Revis Bionatura 2022; 7(1): 29. doi: 10.21931/RB/2022.07.01.29.
16. Mahmood NH, Al-Brefkani A. Molecular Characterisation of Carbapenemase-Producing *Acinetobacter baumannii* isolates from Hospitalised Patients in Iraq. Journal of Life and Bio Sciences Research 2022; 17,3(02):27-32. doi.org/10.38094/jlbsr30261.
17. Al-Kadmy IM, Ali AN, Salman IM, Khazaal SS. Molecular characterization of *Acinetobacter baumannii* isolated from Iraqi hospital environment. New Microbe and New Infect 2018; 1(21):51-7. doi.org/10.1016/j.nmni.2017.10.010.
18. Radhi SH, Al-Charrakh AH. Occurrence of MBLs and Carbapenemases among MDR and XDR *Acinetobacter baumannii* Isolated from Hospitals in Iraq. Indian J Public Health Res Dev 2019;10(7):668.
19. Nimer NA, Al-Saa'da RJ, Abuelaish O. Accuracy of the VITEK 2 system for a rapid and direct identification and susceptibility testing of gram-negative rods and gram-positive cocci in blood samples. East Mediterr Health J 2016; 22(3): 193-200.
20. Clinical and Laboratory Standards Institute (CLSI) Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Fifth Informational Supplement. CLSI document M100-S25 2015.
21. Nordmann P, Poirel L, Carrër A, Toleman MA, Walsh TR. How to detect NDM-1 producers. J Clin Microbiol 2011;49(2):718-21. doi.org/10.1128/JCM.01773-10.
22. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 2011; 70(1):119-23. doi.org/10.1016/j.diagmicrobio.2010.12.002.
23. 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; 43:4382–90. doi.org/10.1128/JCM.43.9.4382-4390.2005.
24. Nguyen M, Joshi SG. Carbapenem resistance in *Acinetobacter baumannii*, and their importance in hospital-acquired infections: a scientific review. J Appl Microbiol 2021;131(6): 2715-38. doi: 10.1111/jam.15130.
25. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, Pulcini C, Kahlmeter G, Kluytmans J, Carmeli Y, Ouellette M. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis 2018; 1, 18(3):318-27. doi:10.1016/S1473-3099(17)30753-3.
26. Moghnieh RA, Kanafani ZA, Tabaja HZ, Sharara SL, Awad LS, Kanj SS. Epidemiology of common resistant bacterial pathogens in the countries of the Arab League. Lancet Infect Dis 2018; 1;18(12):e379-94. doi.org/10.1016/S1473-3099.
27. Khoshnood S, Sadeghifard N, Mahdian N, Heidary M, Mahdian S, Mohammadi M, Maleki A, Haddadi MH. Antimicrobial resistance and biofilm formation capacity among Acinetobacter baumannii strains isolated from patients with burns and ventilator-associated pneumonia. J Clin Lab Anal 2023; 37(1):e24814. doi: 10.1002/jcla.24814.

28. Abouelfetouh A, Torky AS, Aboulmagd E. Phenotypic and genotypic characterization of carbapenem-resistant *Acinetobacter baumannii* isolates from Egypt. Antimicro Resist Infect Control 2019; 8(1):1-9. doi.org/10.1186/s13756-019-0611-6.
29. Lin MF, Lan CY. Antimicrobial resistance in *Acinetobacter baumannii*: from bench to bedside. World J Clin Cases 2014; 2(12):787–814. doi: 10.12998/wjcc.v2.i12.787.
30. Ganjo AR, Maghdid DM, Mansoor IY, Kok DJ, Severin JA, Verbrugh HA, Kreft D, Fatah MH, Alnakshabandi AA, Dlnya A, Hammerum AM. OXA-carbapenemases present in clinical *Acinetobacter baumannii-calcoaceticus* complex isolates from patients in Kurdistan region, Iraq. Microb Drug Resist 2016; 1;22(8):627-37. doi.org/10.1089/mdr.2015.0060.
31. Anoar KA, Ali FA, Omer SA. Detection of metallo [beta]-lactamase enzyme in some gram negative bacteria isolated from burn patients in sulaimani city, Iraq. Eur Sci J 2014; 1;10(3). ISSN: 1857 – 7881 e - ISSN 1857- 7431.
32. Joshi PR, Acharya M, Kakshapati T, Leungtongkam U, Thummeepak R, Sitthisak S. Co-existence of bla OXA-23 and bla NDM-1 genes of *Acinetobacter baumannii* isolated from Nepal: antimicrobial resistance and clinical significance. Antimicrob Resist Infect Control 2017;6(1):1-7. doi 10.1186/s13756-017-0180-5.
33. Zhou S, Chen X, Meng X, et al. ‘‘Roar’’ of blaNDM-1 and ‘‘silence’’ of blaOXA-58 co-exist in *Acinetobacter pittii.* Sci Rep 2015;5:8976. doi: 10.1038/srep08976
34. Roberts PJ, Hassan JS, Li X, Li M. Characteristics of 32 *Acinetobacter baumanni* isolates co-harboring OXA-carbapenemases co-existing with 16S rRNA methylase gene. Afr J Microbiol Res 2016; doi.org/10.5897/AJMR2015.7804.
35. Uzunoglu E, Direkel S, Kocbiyik M, Uludag SK, Cicek AC. Co-existance of isaba1/blaoxa-51/23 is increasing in carbapenem rersistant *Acinetobacter baumannii* isolates in Turkey. Acta Medica Mediterr 2017; 1;33(6):1001. doi:10.19193/0393-6384_2017_6_159.
36. Rafei R, Hamze M, Pailhoriès H, Eveillard M, Marsollier L, Joly-Guillou ML, Dabboussi F, Kempf M. Extrahuman epidemiology of *Acinetobacter baumannii* in Lebanon. Appl Environ Microbiol 2015;1;81(7):2359-67. doi.org/10.1128/AEM.03824-14.
37. Tomaschek F, Higgins PG, Stefanik D, Wisplinghoff H, Seifert H. Head-to-head comparison of two multi-locus sequence typing (MLST) schemes for characterization of *Acinetobacter baumannii* outbreak and sporadic isolates. PLoS One 2016; 12;11(4):e0153014. doi.org/10.1371/journal.pone.0153014.
38. Lee HY, Huang CW, Chen CL, Wang YH, Chang CJ, Chiu CH. Emergence in Taiwan of novel imipenem-resistant *Acinetobacter baumannii* ST455 causing bloodstream infection in critical patients. J Microbiol Immunol Infect 2015; 1; 48(6):588-96. doi.org/10.1016/j.jmii.2015.03.003.