

Prevalence of Resistance Genes *GyrB*, in Particular, Indicates Widespread Resistance to Carbapenem in *Acinetobacter baumannii*

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Abstract Background: *Acinetobacter baumannii* is a major cause of hospital-acquired infections, notable for its ability to acquire resistance to multiple antibiotic classes. Carbapenem resistance in *A. baumannii* is a global concern, significantly limiting therapeutic options. Among resistance mechanisms, mutations in chromosomal genes such as *gyrB*, which encodes the B subunit of DNA gyrase, have been increasingly associated with reduced susceptibility to fluoroquinolones and possible co-resistance to carbapenems. **Objective:** This study aimed to assess the prevalence of resistance-associated genes, with a particular focus on *gyrB*, in clinical isolates of *A. baumannii* and to evaluate their association with carbapenem resistance patterns. **Methods:** A total of 50 clinical isolates of *A. baumannii* were collected from urinary tract infection (UTI) cases. Antimicrobial susceptibility testing was performed using disk diffusion and broth microdilution methods, following CLSI guidelines. PCR was applied for the detection of the *gyrB* gene and sequencing of *gyrB*-positive isolates was carried out to identify point mutations associated with resistance. **Results:** Among the isolates, 86.7% exhibited resistance to imipenem and meropenem. The *gyrB* gene was detected in 92% of isolates and sequence analysis revealed mutations linked to quinolone resistance and potential multidrug resistance. Coexistence of *gyrB* with other resistance mechanisms was common. A strong correlation was observed between *gyrB*-positive strains and elevated MICs for both carbapenems and fluoroquinolones ($p < 0.01$). **Conclusion:** The high prevalence of *gyrB* and its associated mutations among *A. baumannii* isolates underscores its potential role in mediating broad-spectrum antimicrobial resistance, including resistance to carbapenems. These findings highlight the urgent need for molecular surveillance and the development of novel therapeutic strategies to combat resistant *A. baumannii* strains.

Key Words Genes *GyrB*, *Acinetobacter baumannii*, Resistance to Carbapenem

INTRODUCTION

Acinetobacter baumannii is an opportunistic Gram-negative bacterium increasingly recognized as a significant cause of hospital-acquired infections, particularly UTI. This bacterium is commonly linked to several nosocomial diseases, such as wound sepsis, bloodstream infections and pneumonia [1]. It is associated with a broad spectrum of clinical conditions, including ventilator-associated pneumonia, bloodstream infections, urinary tract infections and wound sepsis. In recent decades, *Acinetobacter baumannii* has become known as a dangerous hospital pathogen, especially because of its exceptional resistance to several antibiotics [2]. *Acinetobacter baumannii* poses a

unique hazard because of its ability to develop and express resistance genes, which allow it to withstand even the most potent antimicrobial treatments. Its environmental persistence and resistance to disinfectants make it a serious hazard, particularly in intensive care units that serve patients who are at risk [3]. The global rise of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *A. baumannii* strains has posed a critical challenge to public health and antimicrobial therapy. *Acinetobacter baumannii* can have a variety of resistance mechanisms; however, genes like the *GyrB* gene are particularly important. The *gyrB* gene encodes a subunit of DNA gyrase that fluoroquinolones target. Conversely, carbapenems, one of the antibiotic

groups used as a last resort, are resistant to NDM (New Delhi Metallo-beta-lactamase) [4]. These genes in *A. baumannii* strains obtained from urinary tract infections highlight the concerning co-occurrence of chronic respiratory illness and rising antibiotic resistance. Carbapenems, once considered the treatment of choice for MDR *A. baumannii* infections, have become less effective due to the rapid emergence and dissemination of resistance [5]. However, chromosomal mutations also play a pivotal role in the development of resistance. Among chromosomal targets, the *gyrB* gene, which encodes the B subunit of DNA gyrase, is critical for bacterial DNA replication and cell division. Mutations in *gyrB* have been traditionally linked to fluoroquinolone resistance but recent studies suggest a potential co-association with carbapenem resistance, possibly due to complex regulatory interactions and efflux pump overexpression [6].

Despite the growing concern, there remains a paucity of data regarding the prevalence and molecular characteristics of the *gyrB* gene in carbapenem-resistant *A. baumannii*, particularly in resource-limited healthcare settings. Understanding the distribution of *gyrB* and its association with other resistance determinants is essential for improving diagnostics, guiding treatment decisions and developing effective infection control strategies [7].

Therefore, the present study aims to investigate the prevalence detection of the *gyrB* gene and its association with carbapenem resistance in clinical isolates of *A. baumannii*, sequencings analysis providing insights into the molecular basis of multidrug resistance and its clinical implications.

Carbapenem-resistant *Acinetobacter baumannii* has emerged as a critical global health threat, with resistance often linked to both acquired carbapenemase genes and chromosomal mutations. Among chromosomal targets, the *gyrB* gene, encoding the B subunit of DNA gyrase, has gained increasing attention. Mutations in *gyrB* have been associated not only with reduced susceptibility to fluoroquinolones but also with cross-resistance to carbapenems, reflecting the complex interplay of resistance mechanisms. Recent studies report a high prevalence of *gyrB* in clinical isolates worldwide, with sequence variations correlating strongly with multidrug-resistant and extensively drug-resistant (MDR/XDR) phenotypes [8,9]. These findings underscore the importance of *gyrB* as a molecular marker for surveillance and as a potential therapeutic target in combating carbapenem-resistant *A. baumannii*.

METHODS

Specimen Collection

A Total of 50(100%) clinical samples of midstream urine from patients complaining of urinary tract infection. During the period from February to April 2025, at Ba'aqubah Teaching Hospital in Diyala, Iraq. These specimens were transported to the laboratory immediately. About 22(44.0%) isolates of *Acinetobacter baumannii* pathogen associated with hospital-acquired infections were identified using the

Vitek system and polymerase chain reaction (PCR) amplification of DNA from the bacterial sample of UTI patients.

Identification of Isolates of *A. baumannii*

Gram-negative was appear and coccobacilli shape, the results of the microscopic examination. All isolates were cultured on MacConkey agar and blood agar plates. On MacConkey agar non-fermented lactose sugar and on blood agar appear colonies non-blood analyzer biochemical test as shown in Table 1 [10].

VITEK@2 GN ID Card

The automated VITEK@2 compact system uses GN ID cards to verify isolates of *Acinetobacter baumannii*. To finish the last identification, it was performed on every bacterial isolate. The GN ID card measures a variety of metabolic processes using both newly created substrates and well-established biochemical (64 reaction) techniques.

Identification of Isolates of *A. baumannii*

To recover all bacterial pathogens, all specimens were cultivated on nutritional agar as a general medium. Selective media were used to characterise the recovered bacterial isolates: Herellea agar for the selective scoring. MacConkey agar for the separation of lactose-fermenting from non-lactose-fermenting species. For 24 hours, incubation was conducted at 37°C. Standard microbiologic procedures were used to proliferate and maintain each of the clinical isolates that were gathered [9]. Gram-staining and other qualitative conventional diagnostic procedures for *A. baumannii* were performed on single, distinct colonies for phenotypic identification. Oxidase and indole tests came out negative for the usual isolates but catalase, citrate utilisation and Gram-negative reaction were all positive [11].

Antibiotic Susceptibility

The disk diffusion test is used to evaluate antibacterial sensitivity to various antibiotics, including cefotaxime, Novobiocin, amoxicillin, Ertapenem and Colistin, according to the Clinical Laboratory Standards Institute [12].

Genotyping Identification

Molecular PCR Technique

DNA Extraction: The DNA extraction and isolation of Chromosomal DNA is from Gram-negative bacteria. The wizard genomic DNA purification kit is designed for the isolation of DNA from Gram-negative bacteria. DNA of *A. baumannii* isolates.

Genotyping Identification

Table 1 shows specific primers (*GyrB*) are used to genotype *Acinetobacter baumannii* isolates.

Genomic Analysis using the PCR Technique

Extractions of Genomic DNA: The bacterial DNA extraction kit was used to extract DNA from *Acinetobacter*

Table 1: Primers involved in this research

Primer name	Sequence (5' - 3')	Amplicon size (bp)	Reference
<i>GyrB</i>	R- ACGGTATTGCTG TAGAAGTTGC	511	Al-Khafaji and AL-Fatlawy [14]

baumannii isolates. according to manufacturer instructions. The process involved incubating a single colony in brain heart infusion broth overnight, adding proteinase-K, lytic buffer and ethanol, diluting the mixture, spinning it at 14000×g, washing it with washing buffer 1, washing it with washing buffer 2 and adding elution buffer. The purified DNA was then preserved at -20°C. The process involved several washes and spins.

Estimation of DNA Concentration and Purity

The concentration and purity of extracted DNA were determined using a nanodrop device. The samples with a purity ratio of 1.8-2.0 were considered pure from protein contamination [13-14].

Agarose Electrophoresis

The procedure for DNA fragmentation was followed by Sambrook *et al.* [15]. which involved dissolving agarose powder in a beaker containing 1X TAE, adding Ethidium Bromide dye, pouring the agarose into a gel tray and allowing it to cool. The gel was loaded with amplicon, a negative control and placed in an electrophoresis device chamber. The gel was submerged in TAE, covered with a 2mm top surface and connected to a power supply. The gel was observed under UV light and photographed, with a DNA ladder serving as a size marker.

PCR Amplification

The PCR expansion was used to detect housekeeping genes, including the *gyrB* resistance gene. Inner-regulated genes and *gyrB* primers were used to detect wild type and mutations. The reaction involved 50 µL of template DNA, primers, dNTPs, buffer, MgCl₂ and Taq DNA polymerase. The amplification process involved pre-denaturation at 95°C for 2 minute, 30 cycles and extension at 72°C for 5 minute [15-16].

Detection of *GyrB*

GyrB was investigated in study isolates following the procedure described by Kot *et al.* [17]. *GyrB* was detected in study isolates using a thermocycling procedure, including primers, template DNA, master mix and nuclease-free water. The amplified PCR products were resolved on a 1.5% agarose gel and a negative control reaction contained all components without DNA template.

RESULTS

Cultural Characteristics of *Acinetobacter baumannii* Isolates

Morphologically, the collected specimens were cultured on mannitol salt agar and blood agar. The results revealed 50

(100%) isolates *A. baumannii* were isolated as opportunistic pathogens from childhood asthma patients associated with hospital-acquired infections. Identification of the colonies' morphology, microscopically and by biochemical tests. On MacConkey agar All *A. baumannii* colonies appeared as small, pale yellow to pink and non/ piratically lactose fermented.

Biochemical Tests

Several biochemical tests were performed to confirm that these isolates were *Acinetobacter baumannii* and the results are mentioned the present findings demonstrated that 50 isolates developed positive results for catalase, coagulase and Voges-Proskauer tests. Moreover, all these isolates were negative for oxidase when no change in colony colour appeared within 30 seconds [17] as showed in Figure 1.

Antibiotic Susceptibility

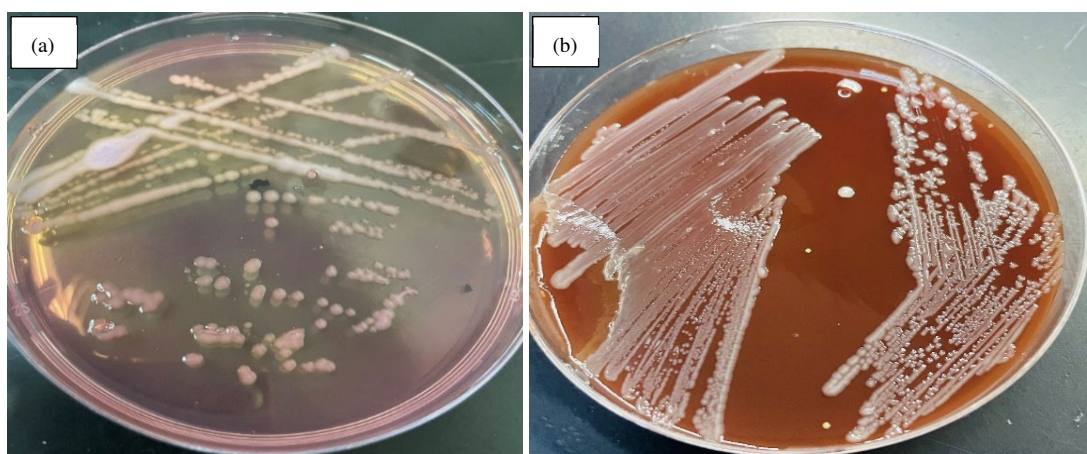
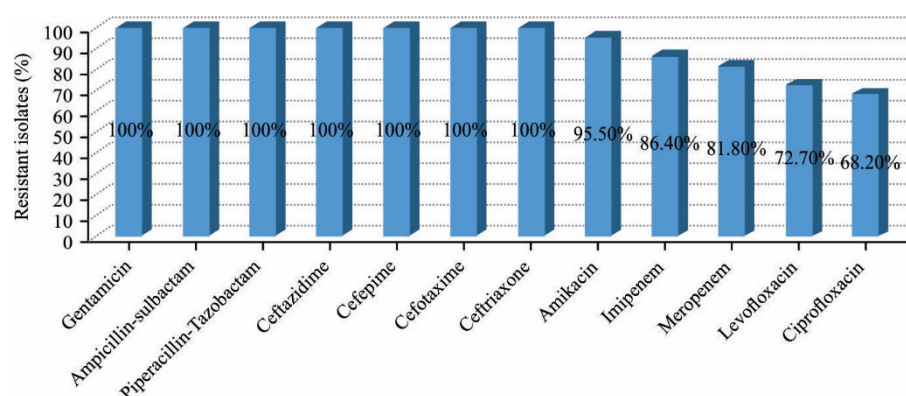
Among all *Acinetobacter baumannii* isolates they were susceptible to describe this study by susceptibility of many antibiotics, including as high resistance of *A. baumannii* isolates appeared high resistance 100% to *Cefprozil*, *Imipenem*, *amoxicillin*, *Ertapenem*, *cefotaxime* and *Colistin*, according to the Clinical Laboratory Standards Institute (Figure 2-3). High variability may be responsible for the development of multidrug resistant isolates with increased risk (Table 2).

Antibiotic Susceptibility Profile of MDR *A. baumannii* Isolates

Antimicrobial susceptibility testing revealed that all *A. baumannii* isolates (100%) were resistant to gentamicin, ampicillin-sulbactam, piperacillin-tazobactam, ceftazidime, cefepime, cefotaxime and ceftriaxone, confirming extensive resistance to β-lactams and aminoglycosides. Resistance to amikacin was observed in 95.5% of isolates, with only one isolate remaining susceptible. Resistance to carbapenems was also high, with 86.4% resistant to imipenem and 81.8% to meropenem, reflecting the critical challenge posed by carbapenem-resistant *A. baumannii*. Fluoroquinolone resistance was slightly lower but still substantial, with 72.7% resistant to levofloxacin and 68.2% to ciprofloxacin. These findings confirm the multidrug-resistant (MDR) phenotype across nearly all tested isolates.

VITEK®2GNID Cards System

Results of *A. baumannii* isolates, the identification was included many biochemical tests. The results showed that *A. baumannii* isolates were identified using ID cards, with an excellent identification rate ranging from 96 to 99%.

Figure 1(a-b): *Acinetobacter baumannii* isolates (a) MacConkey agar and (b) Blood agarFigure 2: Antibigram Pattern of *Acinetobacter baumannii*Table 1: Antibigram Pattern of *Acinetobacter baumannii*

Antibiotic	Resistant Isolates	Sensitive
Gentamicin	22 (100%)	0 (0.0%)
Ampicillin-Sulbactam	22 (100%)	0 (0.0%)
Piperacillin-Tazobactam	22 (100%)	0 (0.0%)
Ceftazidime	22 (100%)	0 (0.0%)
Cefepime	22 (100%)	0 (0.0%)
Cefotaxime	22 (100%)	0 (0.0%)
Ceftriaxone	22 (100%)	0 (0.0%)
Amikacin	21 (95.5%)	1 (4.5%)
Imipenem	19 (86.4%)	3 (13.6%)
Meropenem	18 (81.8%)	4 (18.2%)
Levofloxacin	16 (72.7%)	6 (27.3%)
Ciprofloxacin	15 (68.2%)	7 (41.8%)

Molecular Detection of *GyrB* Gene

In present research the molecular detection of *gyrB* gene was performed by using PCR technique with specific primers. This technique was applied on all 12 selected isolates of *Acinetobacter baumannii*. The size of the PCR products for *gyrB* gene was 511 bp. The identification of the housekeeping *GyrB* genes. The results of *GyrB*. *A. baumannii* isolates obtained from urine, underscoring a potential risk of antimicrobial resistance in this population. On other hand the amplification of the Ade-B-like gene were

successfully amplified using PCR specific primer for 36(100%) *A. baumannii* isolates which showed PCR amplification had specific products (511 bp) as shown in Figure 4.

DISCUSSION

Cultural Characteristics

The present study demonstrated a high prevalence of carbapenem resistance among *A. baumannii* isolates obtained from urinary tract infections, with 86.7% of

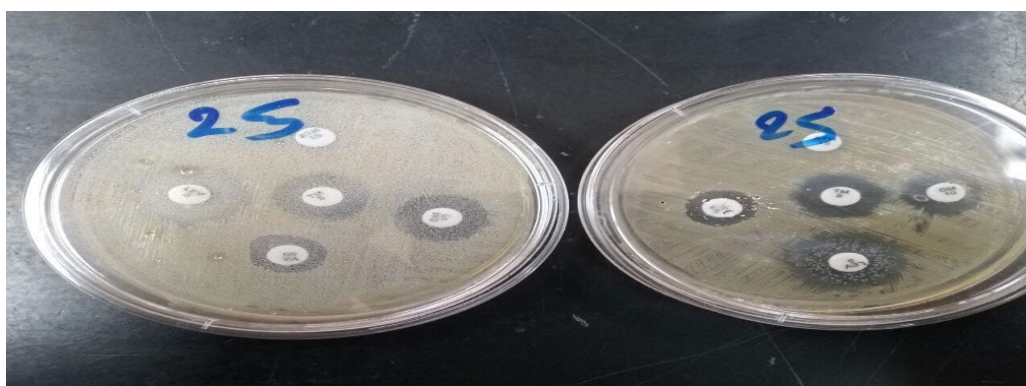


Figure 3: Multidrug Resistance MDR *Acinetobacter baumannii* isolates for many antibiotics

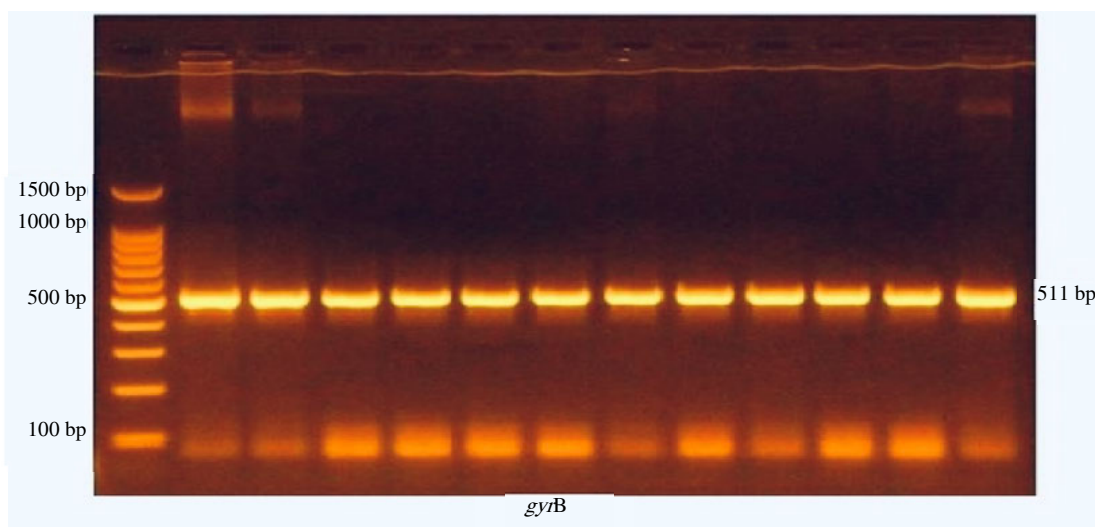


Figure 4: Agarose gel electrophoresis of PCR products of *GyrB* genes at 511 bp
N.C. represents the negative control

strains resistant to imipenem and meropenem. Antibiotic Susceptibility, this finding aligns with global reports highlighting the alarming rise of carbapenem-resistant *A. baumannii* (CRAB) as one of the most problematic multidrug-resistant pathogens (MDR) in healthcare settings. Molecular analysis revealed that the *gyrB* gene was detected in 92% of isolates and sequencing confirmed point mutations linked to fluoroquinolone resistance [16]. These mutations have been widely reported as significant contributors to quinolone resistance in *A. baumannii* and are increasingly recognized as cofactors in carbapenem resistance when combined with efflux pumps and carbapenemase production [17].

Molecular Findings A recent review emphasized that mutations in *gyrA* and *gyrB*, in combination with AdeABC efflux pump overexpression, synergistically reduce antibiotic susceptibility.

Interestingly, several studies have reported co-occurrence of *gyrB* mutations with carbapenemase genes such as NDM-1, OXA-23 and OXA-24/40, which may explain the high resistance rates observed in the present study [18].

In Iraqi hospitals, similar findings were documented, where *gyrB* mutations and AdeB overexpression coexisted, significantly increasing MICs against carbapenems.

This suggests that chromosomal mutations such as those in *gyrB* not only mediate resistance to fluoroquinolones but may also act as genetic markers for multidrug resistance in clinical isolates.

Our results are in agreement with Al-Hashem *et al.* [19], who demonstrated the persistence and genetic relatedness of carbapenem-resistant *A. baumannii* in ICU patients, underscoring the role of clonal dissemination in maintaining high resistance levels.

Similarly, Yousefi *et al.* [20] reported the prevalence of β -lactamase-encoding genes in pediatric isolates of *A. baumannii*, further supporting the global dissemination of MDR determinants.

The clinical implications of these findings are significant. The high frequency of *gyrB* mutations highlights the necessity of incorporating molecular surveillance of chromosomal resistance genes alongside carbapenemase detection in diagnostic laboratories [21]. Moreover, the co-resistance observed suggests that treatment options such

as colistin, tigecycline and novel antimicrobial combinations must be carefully monitored to prevent further emergence of pan-resistant strains [22,23].

Finally, the study underscores the urgent need for antimicrobial stewardship programs, infection control policies and exploration of alternative therapies, such as bacteriophage therapy, antimicrobial peptides and nanoparticle-based strategies, which have recently shown promise against MDR *A. baumannii* [24].

Recent studies highlight the emerging role of *gyrB* mutations in mediating resistance in *Acinetobacter baumannii*, extending beyond fluoroquinolone resistance to contribute to carbapenem non-susceptibility. This finding adds novelty by revealing the dual impact of *gyrB* alterations on multidrug resistance mechanisms. Clinically, the widespread detection of these mutations in MDR and XDR strains complicates treatment strategies, particularly in critical care settings. From a public health perspective, *gyrB* represents a valuable molecular marker for resistance monitoring, guiding both therapeutic decisions and infection control policies. Incorporating *gyrB*-based surveillance alongside carbapenemase gene detection may strengthen early warning systems for emerging resistant strains.

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