Kufa Medical Journal | Vol. 21, No. 1, 2025

Original article | DOI: https://doi.org/10.36330/kmj.v21i1.19690

p-ISSN: 1993-517X
e-ISSN: 2709-4464

# Prevalence of Resistance Genes Gyr B, Ade-B and NDM-1 in *Acinetobacter baumannii* from Asthmatic Children

**Adian Maytham Kazam Al-Khafaji[1] and Hawraa Natiq Kabroot AL-Fatlawy[2]**

[1,2] *Medical Microbiology, Faculty of Medicine, University of Kufa, Iraq.*

**E-Mail:** adianm.sder@student.uokufa.edu.iq[1] hawraan.alfatlawy@uokufa.edu.iq[2]

## ABSTRACT

***Background:*** ***Acinetobacter baumannii*** *is an opportunistic pathogen associated with hospital acquired infections in AL-Zahraa Educational Hospital in Najaf, Iraq. All samples are being collated during the period from November 2024 to the April 2025 including 250 clinical samples from sputum collected from Asthmatic childhood patients with respiratory infections in Najaf/ Iraq. The present study is designed to detect Gyrase B, AdeB and NDM-1, genes strain.* ***Aim of study:*** *To isolate and identify bacterial species isolated from sputum samples of children with asthma and respiratory infections for the purpose of determining the bacterial types associated with or contributing to asthma conditions. As well as to perform molecular detection of housekeeping genes and antibiotic resistance genes such as (NDM-1), which are known to play a major role in carbapenem resistance in Acinetobacter baumannii, also to identify the housekeeping gene (gyrB and Ade-B), and use it as a molecular reference for bacterial identification and comparative genetic analysis among isolates.* ***Results****: Thirty-six of A. baumannii were diagnosed using the VITEK system and molecular detection (PCR) revealed that most isolates of A. baumannii were positive results to the gyrB gene and Ade-B gene (36/36, 100%). The specific primer (gyrB) gene for A. baumannii bacteria was designed in this study by using bioinformatics programs with NCBI website. NDM-1 gene was positive result in 42% of isolates.* ***Conclusion:*** *Presence of multidrug-resistant A. baumannii in childhood asthma patients, with detection of key resistance genes (Ade-B, NDM-1, and gyrB). These findings highlight the serious treatment challenges and the importance of molecular diagnostics and antimicrobial stewardship.*

**Keywords:** ***Acinetobacter baumannii*****, Gyr B gene, Ade-B gene, NDM-1 gene, and**

**Article Information**
**Received: May 15, 2025; Revised: June 12, 2025; Online June, 2025**

## INTRODUCTION

*Acinetobacter baumannii* has gained notoriety in recent decades as a formidable pathogen in hospital settings, particularly due to its remarkable ability to resistance multiple antibiotics. This Gram-negative, opportunistic bacterium is frequently associated with a range of nosocomial infections, including pneumonia, bloodstream infections, and wound sepsis. The particular threat *A. baumannii* in is its capacity to acquire and express resistance genes, enabling it to survive even the most aggressive antimicrobial therapies [1]. Its persistence in the environment and resilience against disinfectants pose a significant threat, especially in intensive care units where vulnerable patients are treated.

Asthma, a chronic inflammatory condition of the airways, affects millions of children worldwide and often leads to recurrent hospitalizations and respiratory distress. In pediatric populations, particularly those with moderate to severe asthma, immune dysfunction and frequent corticosteroid use can alter the respiratory microbiome and increase susceptibility to opportunistic infections, including those caused by drug-resistant pathogens [2]. The frequent hospital visits and compromised airway defenses in asthmatic children create a potential avenue for colonization or infection with resilient organisms like *A. baumannii*.

Among the many resistance mechanisms that *A. baumannii* can harbor, genes such as GyrB, AdeB, and NDM genes are especially significant. The GyrB gene encodes a subunit of DNA gyrase targeted by fluoroquinolones, while AdeB is a component of the Ade ABC efflux pump system that reduces intracellular concentrations of antibiotics. NDM (New Delhi Metallo-beta-lactamase), on the other hand, confers resistance to carbapenems, one of the last-resort antibiotic classes [3] (Evans & Amyes, 2014; Dortet et al., 2014). The presence of these genes in A. baumannii strains isolated from asthmatic children underscores a troubling convergence of chronic respiratory disease and escalating antimicrobial resistance

## METHODS

- **Samples collection:**

A Total of 250 clinical samples from sputum collated from Asthma childhood patients during the period from November 2024 to April 2025 in hospital-acquired respiratory infections in AL-Zahraa Educational Hospital in Najaf,iraq. Thirty six isolate 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 children with asthma infections.

- **CHROM AgarTM Acinetobacter Medium**

The isolates are identified using CHROM agar media, which is specific for Gram-negative bacteria. After the identification by Chrome media. This medium was used for the identification and isolation of *Acinetobacter* spp. (Chromagar, 1996), by streaking on plates, and then incubated at 37˚C for 24 hours. Results showed become *Acinetobacter* and Pseudomonas appeared red color and can easily be differentiated by the oxidase test, Pseudomonas was oxidase positive, while *Acinetobacter* appeared negative.

**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 (BioMérieux Company/http://www.bioMérieux.com).

**Identification of isolates of *A. baumannii.***

To recover all bacterial pathogens, all specimens were cultivated on nutritional agar (Oxide Limited, England) as a general medium. Selective media were used to characterize the recovered bacterial isolates: Herellea agar (Himedia, India) for the selective scoring of *Acinetobacter spp*. MacConkey agar (Oxide Limited, England) 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 [5]. Gramm 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 utilization, and Gram-negative reaction were all positive [6].

**Antibiotic susceptibility**

The disk diffusion test has used to evaluate antibacterial sensitivity to various antibiotics, including cefotaxime, Novobiocin, amoxicillin, Ertapenem, and Colistin, according to the Clinical Laboratory Standards Institute (CLSI,2024) [17].

## 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.

- **Primer design**

The specific primer (gyrB and Ade-B) genes for *A. baumannii* bacteria is designed in this study by using bioinformatics programs with NCBI website.

- **Genotyping identification**

[Table-1]: Specific primers (Gyr B, Ade-B, and NDM-1) are used to genotype *Acinetobacter baumannii* isolates

| No | Primer | Sequence 5-3 | Product Size | Reference |
|---|---|---|---|---|
| 1 | Gyr B | ACGGTATTGCTG TAGAAGTTGC GCGTGTCATTTC ACGTGCTT | 437 | This study |
| 2 | Ade-B | CCGGTATTACCT TTGCCGGA TTGACCGCTTGA ACCCATGA | 728 | This study |
| 3 | NDM-1 | GGTTTGGCGATC TGGTTTTC CGGAATGGCTCA TCACGATC | 621 | [9] |

- **PCR Amplification**

The PCR expansion was done to detect the housekeeping genes, including gyrB and Ade-B, and NDM-1 resistance gene. In addition to the usage of inner-regulated genes, gyrB and Ade-B and NDM-1 primers were used for detecting the wild type and its mutations. The primer and sequences shows in [Table 1].

For amplification of the reaction, 50µl of the mixture contained 1µg of the template DNA, 1µM of each of the primers, 200 µM of dNTPs, 1X buffer, 1.5 mM MgCl2, and 1.5 U of Taq DNA polymerase. The essential circumstances were used for the multiplication: (Gyr B) pre-denaturation at 95°C for 2 min, 30 cycles of amplification (95°C, 50sec, 57°C, 50sec, 72°C, 50sec). Ade-B were used pre-denaturation at 95°C for 5 min, 30 cycles of amplification (95°C, 40sec, 59.8°C, 50sec, 72°C, 50sec), and a final extension at 72°C for 5 min. NDM-1 were used pre-denaturation at 95°C for 2 min, 36 cycles of amplification (95°C, 50sec, 52°C,

50sec, 72°C, 50sec), and a final extension at 72°C for 5 min. Under UV illumination, generated products from PCR toward genes were seen on a 2% agarose gel [10].

**Statistical analysis**

The data were performed and analyzed using bioinformatics software and Microsoft Excel program. The statistical analysis was reported as percentages. The chi-square statistic was used to compare percentages. P-values less than 0.05 were deemed significant

## RESULTS

**Identifection of *Acinetobacter baumannii***

During the study period, 250 clinical sputum samples were collected from resipratory infections with asthamtic childrens patients from November 2024 to April 2025 at Al-Zahraa Teaching Hospital in Najaf, Iraq. Thirty-six *A. baumannii* were isolated as an opportunistic pathogen from childhood asthma patients associated with hospital-acquired infections. Identifiection of the colonies morphology, microscopically and biochemical tests. On macConkey agar All *A. baumannii* colonies appeared as small, pale yellow to pink and non/ partically lactose fermented. But on CHROM agar *A.baumannii* isolates appeared as red, round and smooth colonies at (figure.1).

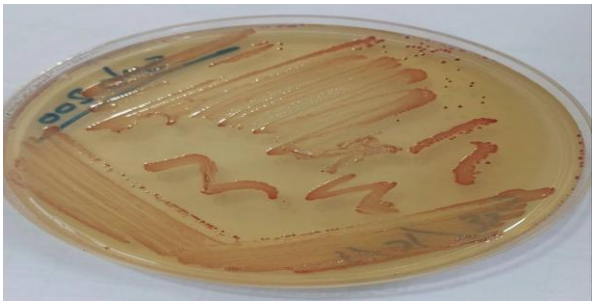


**Figure (1):** *Acinetobacter baumannii* isolate on CHROM agar

**Biochemical tests** results of *A. baumannii* isolates were revealed for Urase, oxidase, simmone citrate, Kligler iron agar and indole test.

[Table-2] : Biochemical tests of *Acinetobacter baumannii* results.

| No | Biochemical tests | *Acinetobacter baumannii* results |
|---|---|---|
| 1 | Urase | Yellew / Yellew |
| 2 | Oxidase | + |
| 3 | Simmone citrate | Blue /blue |
| 4 | Kligler iron agar | Alk/ acid |
| 5 | Indole | + |
| 6 | Motilty | + |

**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%.

**Antibiotic susceptibility**

Among 36 *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, nitrofurantoin, and Colistin, according to the Clinical Laboratory Standards Institute [figure.2]. High variability may be responsible for the development of multidrug-resistant isolates with increased risk.

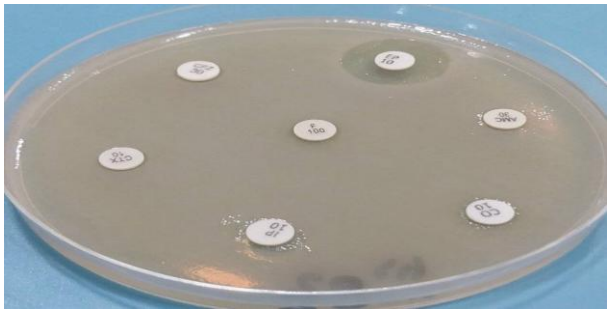


**Figure (2):** Multidrug Resistance MDR *Acinetobacter baumannii* isolates for many antibiotics.

**Molecular Characteristics *Acinetobacter baumannii isolates***

**Characteristics of Gyr B, Ade-B and *NDM*-1 Genes of *Acinetobacter baumannii* isolates**

The present results study indicates that the detection of Gyrase subunit B (Gyr B) gene in *Acinetobacter*, which showed gyrB gene with 36(100%) *A. baumannii* isolates. This unique gene encodes gyrase an essential type ll topoisomerase enzyme responsible for introducing negative supercoils into DNA. Gyrase enzyme plays a crucial role in DNA replication. A specific primer (*gyr B*) was designed in this study the online Bioinformatics program of NCBI web. Polymerase chain reaction (PCR) was used to detect the *(gyr B)* gene of *A. baumannii* are visualized in [Table 1] and [Figures 3 and 4].

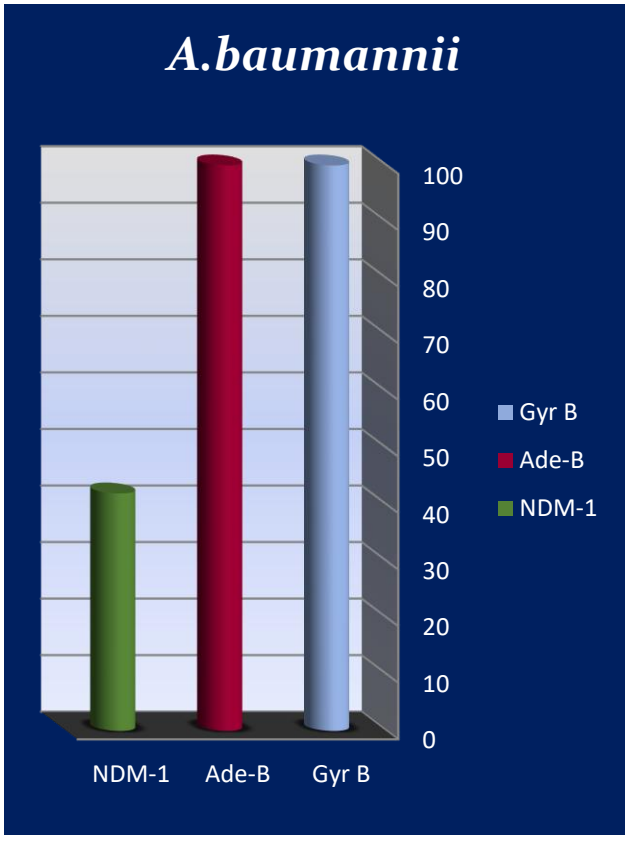



**Figure (3):** prevalence of genes *among A. baumannii* isolated bacteria.

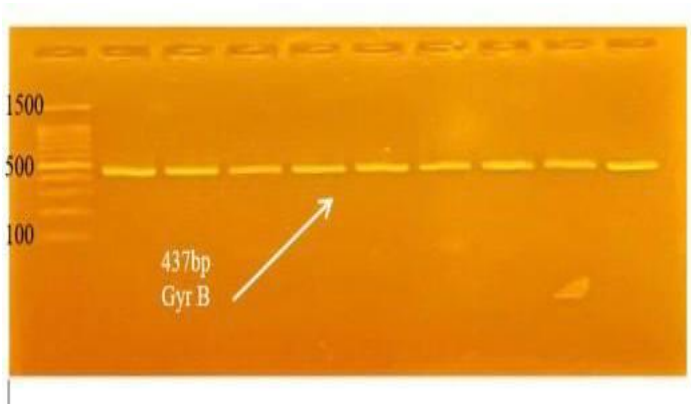
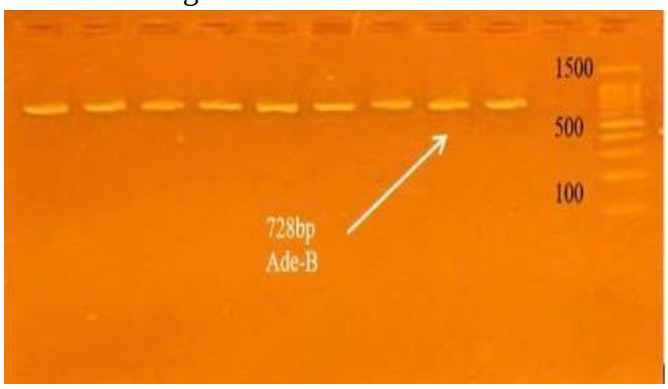



**Figure (4):** The Agarose Gel Electrophoresis (2%) of PCR products of GyrB gene (437 bp ) of *Acinetobacter baumannii* for (45) min at (80) volt.

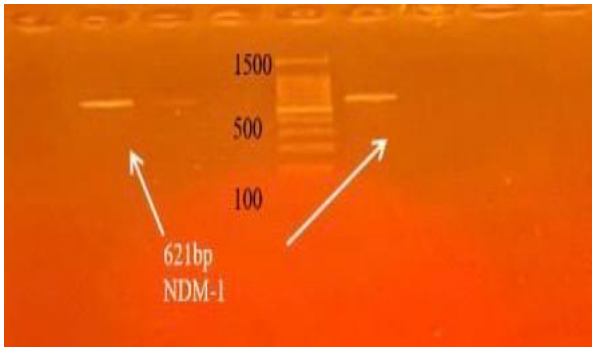
The identification of the housekeeping GyrB and Adeb genes. the results of GyrB and AdeB genes in *A. baumannii* isolates obtained from children with asthma, underscoring a potential risk of antimicrobial resistance in this population. On other hand the amplification of the Ade-B-like gene were successfully amplified using PCR specific primer for 36(100%) *A.baumannii* isolates which showed PCR amplification had specific produts (728bp) as shown in figures.3 and 5.




**Figure(5):** The Agarose Gel Electrophoresis (2%) of PCR products of *Ade-B* gene (728 bp) of *Acinetobacter baumannii* for (45) min at (80) volt.

The detection of the NDM-1 gene in *Acinetobacter baumannii* isolates from childhood asthma patients is a significant finding and it revealed result PCR of NDM-1 gene by (42%) of *A.baumannii* isolates in showed as (figures3 and 6). Underscoring the prevalence of carbapenem-resistant strains in

vulnerable populations. The NDM-1 gene encodes a class D β-lactamase, which confers resistance to carbapenem antibiotics, often considered the last line of defense against multidrug-resistant infections.




**[Figure- 6]:** The Agarose Gel Electrophoresis (2%) of PCR products of *NDM-1* gene (621 bp ) of *Acinetobacter baumannii* for (45) min at (80) volt.

## DISCUSSION

Identification of ***Acinetobacter baumannii*** according on the morphology, biochemical tests, VITEK@2GN ID system and molecular identification. On MacConkey agar, *A. baumannii* typically forms small, smooth, and mucoid colonies that are pale or colorless due to its non-lactose fermenting nature. [4] CHROM agar *A.baumannii* isolates appeared as round and smooth colonies, red color and these colonies may develop a reddish-brown pigmentation over time nature which could be attributed to prolonged incubation or specific strain variations[11]. These characterizes allow it to be involved in numerous nosocomial infections and outbreaks.

It is a strictly aerobic organism [12]. Similarly, a study published by [20] in the Journal of Emerging Infectious Diseases investigated the relationship between glucocorticoid aerosol therapy and *A. baumannii* isolation in patients receiving invasive mechanical ventilation. The study found that treatment with aerosolized glucocorticoids was an independent risk factor for A. baumannii isolation, with a hazard ratio of 1.5 (95% confidence interval: 1.02–2.28; p = 0.038). This suggests that corticosteroid use may increase the risk of A. baumannii colonization or infection in at-risk groups, such as children with asthma [20].

Antimicrobial agents' results were reveled high resistance of *A. baumannii* isolates appeared high resistance 100% to cefotaxime, Novobiocin, amoxicillin, Ertapenem, and Colistin. These agents used strategies were known to significantly reduce the frequency of bacterial infections in childhood asthma patients [13,19]. Among which fluoroquinolones (FQs) have the widest use and FQs in the last forty years had shown good activity against *A. baumannii* isolates in Iraq. However, resistance to these drugs has rapidly emerged [14]. FQs are wi dely prescribed medication in Iraq. The developing resistance of *A. baumannii* to antimicrobial agents has been described and this was attributed to the abundance of these antibiotics in multiple pharmaceutical markets [15]. Many acquired resistance mechanisms have been reported for this pathogen and therefore, render it able to express MDR, XDR, or PDR phenotypes that were associated with significant morbidities and mortalities [16,17,18].

The molecular characterization of *A. baumannii* isolates in this study revealed a strikingly high prevalence of resistance genes, particularly GyrB and AdeB, which were both detected in 100% of the examined strains. The universal presence of GyrB highlights the organism's strong potential for fluoroquinolone resistance, as mutations in the DNA gyrase subunit B are a well-documented mechanism conferring reduced susceptibility to these agents [21]. Similarly, the detection of AdeB in all isolates suggests the activation of the Ade ABC efflux pump system, a well-known contributor to multidrug resistance in A. baumannii [22]. These efflux pumps actively expel a broad range

of antibiotics from the bacterial cell, reducing intracellular concentrations and thereby compromising treatment efficacy.

The relatively lower prevalence of the NDM-1 gene (42%) in this cohort is noteworthy, given its role in encoding New Delhi Metallic-β-lactamase, an enzyme that hydrolyzes nearly all β-lactam antibiotics, including carbapenems. Although not dominant in this sample, the presence of NDM-1 even in a minority of isolates raises significant concern due to its association with rapid horizontal gene transfer and the potential for outbreaks of highly resistant strains [23].

A study conducted by [24] in pediatric units found a similar trend, with higher frequencies of efflux pump and fluoroquinolone resistance genes compared to carbapenems genes, reflecting the evolving resistance landscape in clinical settings.

Other regional studies have reported comparable findings. In a molecular surveillance study from Iraq,[25] demonstrated that AdeB and GyrB were more consistently expressed in A. baumannii isolates compared to carbapenems genes, suggesting a predominant role of chromosomal mutations and intrinsic mechanisms over acquired β-lactamases in certain patient populations. Moreover, the co-occurrence of these genes is often associated with increased virulence and biofilm formation, further complicating clinical management [26].

Prevalence of the GyrB Gene in Acinetobacter baumannii Isolates from Asthmatic Children, the molecular analysis confirmed the widespread presence of the GyrB gene among all tested A. baumannii isolates from asthmatic pediatric patients. The clear and consistent amplification patterns observed on the agarose gel electrophoresis strongly suggest a 100% prevalence of this fluoroquinolone resistance-associated gene within the studied population. This is particularly concerning given the pivotal role of DNA gyrase subunit B (GyrB) in mediating resistance to quinolone-class antibiotics through target site mutations [27].

The ubiquity of GyrB among isolates aligns with several previous studies. For instance,[28] reported a similarly high detection rate of GyrB in multidrug-resistant A. baumannii strains isolated from pediatric intensive care units in India, suggesting a global trend of fluoroquinolone resistance in nosocomial settings. Another study by [29] also confirmed the dominance of GyrB in resistant clinical strains, highlighting its role as a core resistance determinant rather than a sporadic resistance trait.

Furthermore, GyrB mutations often occur in conjunction with mutations in GyrA and overexpression of efflux pumps such as Ade ABC, leading to compounded resistance phenotypes that severely limit treatment options [30]. This multifactorial resistance strategy renders fluoroquinolones like ciprofloxacin increasingly ineffective, especially in vulnerable pediatric populations with underlying conditions such as asthma, where frequent hospitalizations and antibiotic exposure may contribute to selective pressure. The observed high prevalence of the AdeB gene in Acinetobacter baumannii isolates from asthmatic children is consistent with findings from several previous studies, indicating the gene's widespread distribution and clinical relevance.

The overexpression of the Ade ABC efflux pump system, where AdeB functions as the main transporter protein, has been widely implicated in resistance to a broad range of antibiotics and is often upregulated in clinical isolates exposed to antimicrobial pressure:

**Frequent antibiotic exposure:** Asthmatic children may undergo frequent antibiotic treatment due to respiratory infections, creating selective pressure that favors the persistence of resistant strains.

**Nosocomial acquisition:** A. baumannii is a common hospital-acquired pathogen; thus, children in clinical settings are at higher risk of colonization or infection with multidrug-resistant strains.

**Genetic adaptability**: The Ade ABC efflux system is easily upregulated through mutations or insertion sequences, which may explain the widespread presence of the AdeB gene.

Supported those studies by [31] about role of efflux pumps—particularly the resistance-nodulation-division (RND) family—in mediating antibiotic resistance in Acinetobacter species. The authors focused on the Ade ABC efflux system, highlighting its capacity to expel a broad range of antibiotics, including aminoglycosides, β-lactams, and fluoroquinolones, and [32] about the prevalence of the Ade A, AdeB, and Ade C efflux pump genes among multidrug-resistant (MDR) Acinetobacter baumannii isolates collected from clinical settings. Using PCR analysis, the researchers reported a high occurrence of all three genes, with AdeB being the most consistently detected.

**The identification of the NDM-1 gene of *A. baumannii*** isolates from asthmatic children with respiratory infections is consistent with several regional studies suggesting a localized or emerging occurrence of this resistance factor. NDM-1 is a powerful β-lactamase enzyme that hydrolyzing carbapenems, posing a serious challenge to antimicrobial treatment.

**Limited carbapenem exposure in pediatric asthma patients:** Children with asthma may not frequently receive carbapenems, thus exerting less selective pressure for the acquisition or retention of the NDM-1 gene.

**Geographical variability:** The prevalence of NDM-1 varies widely across regions and institutions. Its lower detection in this cohort could reflect regional differences in antibiotic use or infection control practices.

**Horizontal gene transfer limitations**

The NDM-1 gene is often plasmid-borne, and its transfer efficiency among A. baumannii strains may be lower in specific settings or hosts**.**

Supported that several studies, such as those by [33] about Limited Carbapenem Exposure in Pediatric Asthma Patients, where Children with asthma are less likely to be exposed to carbapenems, reducing the selective pressure for acquiring the NDM-1 gene. This limited exposure may contribute to the lower prevalence of NDM-1 in pediatric populations, and according to [34], about Geographical Variability in NDM-1 Prevalence, the prevalence of NDM-1 varies across different regions and institutions, influenced by factors such as antibiotic usage patterns and infection control practices. Lower detection rates in certain cohorts may reflect these regional differences and lastly, by [35][36], the NDM-1 gene is often plasmid-borne, but its transfer efficiency among A. baumannii strains can be limited due to factors like plasmid incompatibility or chromosomal integration, which may hinder its dissemination in specific settings

## CONCLUSION

This study highlights the microbiological significance of *Acinetobacter baumannii* in children with asthma patients, demonstrating its potential involvement in respiratory infections and antibiotic resistance. The identification of resistance-associated geneNDM-1, through PCR confirms the presence of multidrug-resistant *A. baumannii* strains and in particular, indicates widespread resistance to carbapenem. The identification housekeeping of the gyrB and AdeB genes showed highlights results with using molecular diagnostic tools enable accurate species confirmation and detailed resistance profiling, highlights their value in the management pediatric respiratory infections.

These findings emphasize the urgent need for stringent infection control measures and careful antibiotic stewardship to reduce the risk of treatment failure and further spread of resistant strain, particularly in vulnerable children with underlying respiratory conditions like asthma.

## ACKNOWLEDGMENT

We would like to thank to Molecular Biology Laboratory for Postgraduate of department of Medical Laboratory, Faculty of Medicine, University of Kufa, and AL-Zahraa Educational Hospital in Najaf, IRAQ.

State the contributing parties or institutions which help the author's research. It is important to acknowledge those who help the authors in **funding**, **research facilities**, or **meaningful suggestions** in improving the author's article. If the

### Ethical approval

M.Sc. student, **Adian Maytham Kazam Al-Khafaji** Submitted the study was approved by faculty of Medicine, University of Kufa/ Iraq. Supported by Dr. Hawraa Natiq Kabroot AL-Fatlawy.

### Conflict of interest

The authors declare that there is no conflict of interest.

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