Original Article

Iraqi National Journal of Medicine. January 2025, Volume 7, Issue 1

Distribution of resistance genes among carbapenem-resistant *Acinetobacter baumannii* isolated from different clinical specimens

Ghufran Rajab Taher, Ahlam Kadhum Naeem

Department of Biology, Faculty of Education for Women, University of Kufa, Najaf, Iraq.

ABSTRACT

Background: Carbapenems are the drugs of choice for serious hospital-acquired infections and for patients in intensive care unit affected by multidrug-resistant microorganisms, particularly *Acinetobacter baumannii.* Carbapenem-resistant *Acinetobacter baumannii* (CRAB) poses a significant threat to public health. **Aim**: The study aims to focus the distribution of carbapenemase-encoding genes belonging to classes A and B (*bla$_{GES}$, bla$_{KPC,}$* and *blaNDM)* among CRAB isolated from various clinical specimens. **Methods**: A total of 55 bacterial isolates were collected from different clinical specimens and underwent identification using Chrom agar and the Vitek 2 compact (ID) system. The checkerboard technique, modified Hodge test, and Vitek 2 compact (AST) system were employed to detect CRAB. PCR techniques were used to identify the presence of *bla$_{GES}$, bla$_{KPC,}$* and *bla$_{NDM}$*. **Results**: Of the 55 isolates, 43 (78.1%) were identified as *A. baumannii*, with 39 (90.6%) of these being CRAB. All CRAB isolates could produce carbapenemase. The percentages of CRAB isolates possessing *bla$_{GES}$, bla$_{KPC,}$* and *blaNDM were 28.2%, 15.4%,* and 48.7%, respectively. In contrast, 25%a percentage of non-CRAB isolates possessing blaKPC and *bla$_{NDM}$* had 25% of each. **Conclusion**: A high prevalence of CRAB capable of producing carbapenemase and harboring *bla$_{GES}$, bla$_{KPC,}$* and *bla$_{NDM}$* genes belonging to classes A and B was observed among various infections in Al Najaf hospitals.

Keywords: *Acinetobacter baumannii*, carbapenem resistance, carbapenemase, PCR.

Corresponding author: Ghufran Rajab Taher. E-mail: g.matrix1986@gmail.com

Disclaimer: The authors declare no conflict of interest.

DOI: https://doi.org/10.37319/iqnjm.7.1.15

Received: 16 JULY 2024 Accepted:30 AUG 2024 Published online: 15 JAN 2025

INTRODUCTION

Acinetobacter baumannii, a Gram negative coccobacilli, is a significant opportunistic pathogen associated with various hospital-acquired infections, particularly in immunocompromised individuals and critically ill patients with predisposing risk factors such as those in intensive care units, patients on ventilators, and those undergoing surgical interventions or invasive procedures, and the presence of indwelling devices (e.g., catheters, drainage or endotracheal tubes, and artificial implants) also increases the risk of infection, although community-acquired cases occur to a lesser extent.[1] Carbapenems are considered the primary

therapeutic choice for combating infections caused by multidrug-resistant *A. baumannii*. However, there has been a notable increase in carbapenem resistance among clinical *A. baumannii* strains, particularly in regions such as Europe, Latin America, Asia, and Australia.[2] Infections caused by CRAB have been linked to prolonged stays in intensive care units (ICUs), increased healthcare costs, and antibiotic use.[3] Furthermore, CRAB is regarded as a significant pathogen responsible for hospital-acquired infections (HAIs), raising concerns about the potential for pan-drug resistance and outbreaks.[4] Many clinical isolates of *A. baumannii* associated with nosocomial infections exhibit resistance to most major classes of antimicrobial agents, including aminoglycosides, β-lactams, and fluoroquinolones. The emergence of carbapenem-resistant *A. baumannii* isolates has been reported globally, with most of these isolates harboring carbapenem-hydrolyzing ß-lactamase genes. CRAB isolates typically show resistance to commonly used antibiotics.[5] They possess multiple virulence genes that enhance their ability to cause acute infections in both animals and humans.[6] Several factors including the K1 surface antigen protein 1, capsular polysaccharides, acinetobactin transporters, outer membrane porins, and iron acquisition mechanisms, along with acquired antibiotic resistance, have contributed to the rise of *A. baumannii* as a prominent nosocomial pathogen.[7] Resistance mechanisms, including antibiotic-hydrolyzing enzymes, efflux pumps, target modification, and porin deficiency, often work synergistically. The primary mechanism of carbapenem resistance in *A. baumannii* is the inactivation or enzymatic hydrolysis of carbapenems by carbapenemases, specifically OXA-type and metallo-β-lactamases (MBL).[8] Oxacillinases (OXAs) are the most common cause of carbapenem resistance in this species.

The aim of this research is to investigate the prevalence of carbapenemase-encoding genes belonging to classes A and B (KPC, GES, NDM) among CRAB isolated from various clinical specimens.

MATERIALS AND METHODS

Bacterial Isolates

A total of 55 clinical specimens, including sputum, urine, blood cultures, wounds, and burns, were collected from microbiological laboratories in four distinct hospitals located in Al-Najaf Alashraf City between September 2023 and November 2023. The specimens were cultured on Blood Agar Base and MacConkey agar and incubated at 40°C for 24 hours for the primary isolation of bacterial isolates belonging to *A. baumannii*. Suspected bacterial isolates were further cultured on Chrome agar for purification and primary identification of *A. baumannii* and incubated at 37°C for 24 hours.[9]

Identification and Antimicrobial Susceptibility Testing

Vitek 2 Compact system was utilized to confirm the identification of *A. baumannii* and to determine the antibiotic susceptibility patterns of the bacterial isolates.

The Checkerboard Technique for Detection of Carbapenem Resistance in *A. baumannii*

The screening for metallo-beta-lactamase (MBL) production was conducted for all isolates using imipenem (10 µg) and meropenem (10 µg) discs (Himedia) via the Kirby Bauer disc diffusion method. Isolates that yielded positive results in the screening test were further tested using the Imipenem-EDTA Combined Disc test to confirm the results.[10] Briefly, plates of Muller Hinton Agar (MHA) were inoculated with the tested bacterial isolates using the lawn culture technique. An Imipenem disc (10 µg) was placed on the surface of the inoculated MHA plate, and another disc containing a mixture of Imipenem and EDTA (the Imipenem-EDTA disc, 10 µg) was placed 20 mm away from the first disc. The plates were incubated at 37 ºC for 18–24 hours. The inhibition zone of the Imipenem-EDTA combination disc was compared with that of the Imipenem disc alone; if the zone increased by 5 mm, the isolates were classified as MBL-producing isolates.

The Modified Hodge Test

This method, as described by,[11] involved preparing a bacterial suspension of *E. coli* 25922 (equivalent to the 0.5 McFarland standard) and using it to inoculate plates of MHA. An imipenem disk (10 µg, Oxoid) was placed on the surface of the inoculated MHA plates. Selected colonies of the tested *A. baumannii* isolates (2-5 colonies) were cultured in a linear manner starting at the periphery of the disk. The plates were incubated at 35°C for 16 to 20 hours. A carbapenemase-positive result was indicated by enhanced growth exhibiting a clover-leaf indentation.

Polymerase Chain Reactions

DNA extraction of all CRAB and non-CRAB isolates was performed using boiling methods as previously described.[12] The extracted DNA was stored in TE buffer at -80°C until use.

Monoplex PCR techniques were employed to detect *bla*$_{GES}$, *bla*$_{KPC}$, and *bla*$_{NDM}$ using the primer sets listed in Table 1. The PCR reaction consisted of 25 µL of PCR Master Mix (2x PCR MIX Taq Polymerase, GOS BIO, China), 2 µL of each forward and reverse primer (final concentration of each primer was 10 µM/µL), 4 µL of DNA template, and the final volume was completed to 50 µL by adding 17 µL of DNase/RNase-free water. After a brief spin of the PCR mixture, PCR amplification was performed in a thermocycler (QLS, UK) with an initial denaturation at 94°C for 3 minutes, followed by 30 cycles of 94°C for 30 seconds, 55.6°C for 30 seconds, and 72°C for 1 minute; a final extension was performed at 72°C for 8 minutes. The same PCR amplification conditions for *bla*$_{KCP}$ and *bla*$_{NDM}$ were used, except the annealing temperatures were 56.7°C for 30 seconds and 50.3°C for 30 seconds, respectively. Agarose gel electrophoresis was conducted to detect the amplicons using 1.5% agarose gel stained with 5 µL of 0.5 µg/mL Ethidium Bromide. Gel electrophoresis was carried out at 90 Volts/cm for 40min. DNA ladder (100 bp-1500 bp, GOS BIO, China) was used. A gel documentation system was employed to photograph the ethidium bromide-stained agarose gel (Cleaver, UK).[13]

Table1: The sequences of oligonucleotides used in this study

Bla Genes	Sequence 5′→ 3′	Size (bp)	Reference
bla$_{GES}$	F- ATGCGCTTCATTCACGCAC R- TATTTGTCCGTGCTCAGGA	863	(14)
bla$_{KPC}$	F- ATGTCACTGTATCGCCGTCT R- TTACTGCCCGTTGACGCCCA	881	(15)
bla$_{NDM}$	F- GGCCGTATGAGTGATTGC R- GAAGCTGAGCACCGCATTAG	825	

RESULTS

The results of primary identification of *A. baumannii* using Chrome agar (Figure 1) and the Vitek 2 compact system indicated that out of 55 bacterial isolates, 43 (78.1%) were identified as *A. baumannii* (Figure 2).

The results of the checkerboard technique for detecting carbapenem resistance in *A. baumannii* showed that 39 (91%) isolates were CRAB, as indicated by the occurrence of an inhibition zone around the imipenem-EDTA disc compared to the imipenem disc alone (Figure 3), while 4 (9%) isolates were non-CRAB. Additionally, the results of the Modified Hodge Test revealed that all CRAB isolates exhibited the ability to produce carbapenemase (Figure 4).

Genetic Detection of bla Genes

The results of agarose gel electrophoresis of amplicons from the amplification of blaGES, blaKPC, and blaNDM indicated that out of 39 CRAB isolates, 11 (28.2%),11 (28.2) 6 (15.4%), and 19 (48.7%) isolates were carrying these genes, respectively, as evidenced by the appearance of amplicons with molecular weights of 863 bp, 881 bp, and 825 bp (Figures 5, 6, and 7). In contrast, only 1(25%) isolate of non-CRAB possessed blaKPC, and 1 (25%) isolate possessed blaNDM (Table 2).

On the other hand, the genetic pattern of bla genes among CRAB and non-CRAB isolates showed a low percentage of distribution of bla genes among isolates, with a higher percentage of distribution occurring in isolates harboring blaGES and blaNDM, followed by isolates possessing blaKPC and blaNDM, which were 12.8% (5 isolates) and 7.7% (3 isolates), respectively. No non-CRAB isolates possessed more than one bla gene (Table 3).

Figure 1: the morphological characters of *Acinobacter baumannii* on chrome agar media

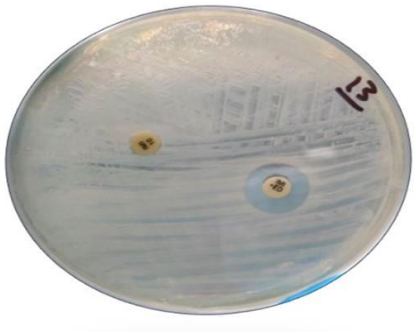

Figure 3: Detection of carbapenem resistance of *Acinobacter baumannii* using checkerboard technique

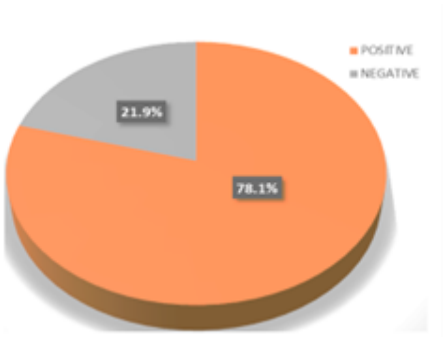

Figure 2: The percentage of *Acinobacter baumannii* isolated from different clinical specimens

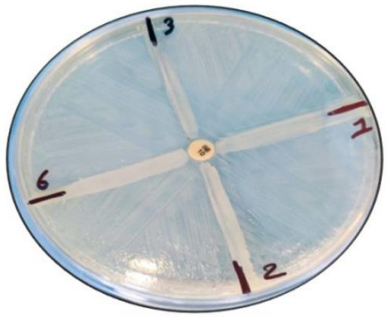

Figure 4: The modified Hodge test of carbapenemase producing *Acinobacter baumannii*

Table 2: The percentage of *Acinetobacter baumannii* isolates carrying *bla* genes

Bacteria	Total No. of Isolates	No. (%) of isolates that carry:		
		bla_{GES}	bla_{KPC}	bla_{NDM}
Carbapenem Resistance *A. baumannii*	39	11(28.20)	6 (15.4)	19 (48.7)
Non-Carbapenem Resistance *A. baumannii*	4	0 (0.0)	1 (25)1 (25)1 (25)	1(25)

Figure 5: Agarose gel electrophoresis of bla_{GES} (863 bp) in *Acinobacter baumannii* isolates

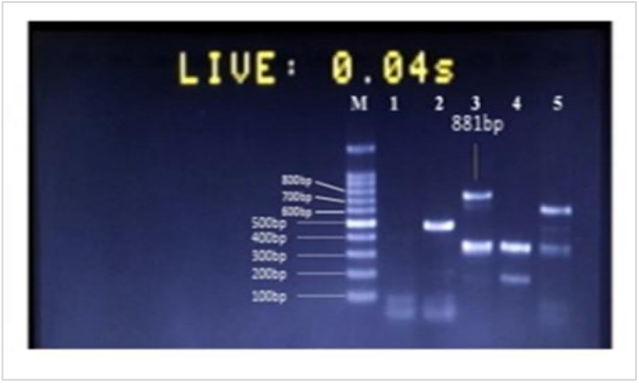

Figure 6: Agarose gel electrophoresis of bla_{KPC} (881 bp) in *Acinobacter baumannii* isolates

Figure 7: Agarose gel electrophoresis of bla_{NDM} (863 bp) in *Acinobacter baumannii* isolates

Table 3: The percentage of *bla* gene patterns in *A. baumannii*

Bacteria	Total No.	NO.(%) of isolates that possess				
		All *bla* genes	*blaGES and* bla_{KPC}	bla_{GES} and bla_{NDM}	bla_{KPC} and bla_{NDM}	One *bla* gene only
Carbapenem Resistance *A. baumannii*	39	0 (0)	0 (0)	5 (12.8)	3 (7.7)	28 (71.8)
Non-Carbapenem Resistance *A. baumannii*	4	0 (0)	0 (0)	0 (0)	0 (0)	1(25)

DISCUSSION

Infections caused by Acinetobacter baumannii present a significant challenge for clinicians and infection-control practitioners worldwide due to *the organism's* ability to thrive in the harsh environments of ICUs, where there is substantial selection pressure from antibiotics and disinfectants. This pathogen has been associated with greater resistance to antibiotics and higher mortality rates among bacteremic patients compared to other genomic species.[16]

Several virulence factors and environmental persistence mechanisms contribute to its extensive antimicrobial resistance and rapid acquisition of antimicrobial-resistance genes (17). Enzymatic hydrolysis by carbapenemases is the most common mechanism of carbapenem resistance in *A. baumannii*, particularly OXA-type and metallo-β-lactamases (MBL). Other mechanisms associated with carbapenem resistance in *A. baumannii* include alterations in penicillin-binding proteins due to downregulation, which results in decreased drug affinity.[18]

CONCLUSIONS

A high prevalence of CRAB capable of producing carbapenemase and harboring *bla$_{GES}$, bla$_{KPC,}$* and *blaNDM genes belonging* to classes A and B was observed among various infections in Al najaf hospitals.

Acknowledgment

We would like to thank Kufa University, Faculty of Education for Women, Department of Biology, and Faculty of Medicine, Department of Microbiology for providing access to the microbiological laboratory.

limitation of study

This study was conducted solely in Najaf province, Iraq, with a limited sample size, which may provide insufficient information about the sources of infection.

REFERENCES

1. Roy S., Chowdhury G, Mukhopadhyay AK, Dutta S, Basu S. Convergence of biofilm formation and antibiotic resistance in Acinetobacter baumannii Infection. Front Med. 2022;9:793615. DOI: 10.3389/fmed.2022.793615
2. Nordmann P, Poirel LJCID. Epidemiology and diagnostics of carbapenem resistance in Gram-negative bacteria. Clin Infect Dis. 2019; 69(7):S521-S528. DOI: 10.1093/cid/ciz824
3. Ejaz H, Ahmad M, Younas S, Junaid K, Abosalif KOA, Abdalla AE. Molecular epidemiology of extensively-drug resistant Acinetobacter baumannii sequence type 2 co-harboring bla NDM and bla OXA from clinical origin. Infect Drug Resist. 2021;1931-39. DOI: 10.2147/IDR.S310478
4. Fournier PE, Richet H, Weinstein RAJC. The epidemiology and control of Acinetobacter baumannii in health care facilities. Clin Infect Dis 2006; 42(5):692-99. DOI: 10.1086/500202.
5. Ramirez MS, Bonomo RA, Tolmasky MEJB. Carbapenemases: Transforming Acinetobacter baumannii into a yet more dangerous menace.
Biomolecules. 2020; 10(5): 720. DOI: 10.3390/biom10050720
6. Harding CM, Hennon SW, Feldman MF. Uncovering the mechanisms of Acinetobacter baumannii virulence. 2018; 16(2):91-102. Nat Rev Microbiol.
DOI: 10.1038/nrmicro.2017.148.
7. Sheldon JR, Skaar EPJ. Acinetobacter baumannii can use multiple siderophores for iron acquisition, but only acinetobactin is required for virulence.
PLoS Pathog. 2020;16(10), e1008995. DOI: 10.1371/journal.ppat.1008995.
8. Abbott I, Gustavo MC, Saruar B, Anton YP. Carbapenem resistance in Acinetobacter baumannii: laboratory challenges, mechanistic insights, and therapeutic strategies. Expert Rev. Anti-Infect Ther. 2013; 11(4):395-409. DOI: 10.1586/eri.13.21.
9. Selvaraj A, Valliammai A, Sivasankar C, Suba M, Sakthivel G, Pandian SKJSR. Antibiofilm and antivirulence efficacy of myrtenol enhances the antibiotic susceptibility of Acinetobacter baumannii. Sci Rep. 2020; 10(1):21975. DOI: org/10.1038/s41598-020-79128-x
10. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong YJ. (2002). Imipenem-EDTA disk method for differentiation of metallo-β-lactamase-producing clinical isolates of Pseudomonas spp. and Acinetobacter spp. Clin Microbiol. 2002; 40(10):3798-3801. DOI: 10.1128/JCM.40.10.3798-3801.2002.
11. Lee K, Chong Y, Shin H, Kim Y, Yong D, Yum JJCM. Modified Hodge and EDTA-disk synergy tests to screen metallo-β-lactamase-producing strains of Pseudomonas and Acinetobacter species. Clin Microbiol Infect. 2001; 7(2): 88-91. DOI: 10.1046/j.1469-0691.2001.00204.x
12. Ranjbar R, Afshar D. Evaluation of (GTG) 5-PCR for genotyping of Klebsiella pneumonia strains isolated from patients with urinary tract infections. Iranian Journal of Public Health. 2019;48(10).
13. Amudhan S, Sekar U, Arunagiri K, Sekar BJIJ. OXA beta-lactamase-mediated carbapenem resistance in Acinetobacter

baumannii. Indian J Med Microbiol. 2011; 29(3):269-274. DOI: 10.4103/0255-0857.83911.

14. Moubareck C, Brémont S, Conroy MC, Courvalin P, Lambert TJA. GES-11, a novel integron-associated GES variant in Acinetobacter baumannii. Antimicrob Agents Chemotherapy. 2009; 53(8): 3579-3581. DOI: 10.1128/AAC.00072-09.
15. Soudeiha MA, Dahdouh EA, Azar E, Sarkis DK, Daoud ZJF. In vitro evaluation of the colistin-carbapenem combination in clinical isolates of A. baumannii using the checkerboard, Etest, and time-kill curve techniques. Front Cell Infect Microbiol. (2017);7:209.
DOI: 10.3389/fcimb.2017.00209.
16. Chuang YC, Sheng WH, Li SY et al. Influence of genospecies of Acinetobacter baumannii complex on clinical outcomes of patients with Acinetobacter bacteremia. Clin Infect Dis. 2011;52(3):352-360. DOI: 10.1093/cid/ciq154.
17. Peleg AY, Seifert H, Paterson DL. Acinetobacter baumannii: Emergence of a successful pathogen. Clin Microbiol Rev. 2008;21:538-582.
DOI: 10.1128/CMR.00058-07.
18. Almasaudi SB. Acinetobacter spp. as nosocomial pathogens: epidemiology and resistance features. Saudi J Biol Sci. 2018;25:586-596. DOI: 10.1016/j.sjbs.2016.02.009