

The Role of CarbAcineto NP Test and blaOXA-51 Gene Detection in Characterizing Carbapenem-Resistant *Acinetobacter baumannii*.

Mahenaz Khan¹, Dr. Ramanath K²

¹Research Scholar, Department of Microbiology, Index Medical College Hospital & Research Centre, Malwanchal University, Indore, Madhya Pradesh- 452016

²Professor, Department of Microbiology, Index Medical College Hospital & Research Centre, Malwanchal University, Indore, Madhya Pradesh- 452016

ABSTRACT

BACKGROUND: The increasing prevalence of carbapenem-resistant *Acinetobacter baumannii* (CRAB) poses a significant threat in healthcare settings, necessitating rapid and accurate detection methods. Phenotypic assays like the CarbAcineto NP test offer quick diagnostic support, while molecular techniques, such as PCR for the *blaOXA-51* gene, provide precise identification of resistance mechanisms and species confirmation. This study investigates the combined utility of these methods in characterizing CRAB isolates from invasive clinical specimens.

OBJECTIVES:

- 1 To evaluate the performance of the CarbAcineto NP test in detecting carbapenemase activity in clinical *A. baumannii* isolates.
- 1 To confirm the presence of the *blaOXA-51* gene as an intrinsic species marker in all *A. baumannii* isolates.
- 2 To assess the diagnostic value of integrating phenotypic and molecular approaches for timely and targeted therapy against CRAB.

METHODOLOGY: A cross-sectional study was conducted involving 123 non-duplicate invasive *A. baumannii* isolates from blood and endotracheal aspirates. Carbapenemase production was assessed using the CarbAcineto NP test. Molecular analysis was performed using PCR to detect the *blaOXA-51* gene, along with other OXA-type and MBL-type carbapenemase genes.

RESULTS: Among the 123 *A. baumannii* isolates, 91.1% (n=112) were identified as carbapenemase producers by the CarbAcineto NP test, with a statistically significant association with sample type ($p = 0.032$). Rapid color change within 15 minutes was observed in 11.3% (n=14) of isolates, while 79.6% (n=98) showed positivity after 2 hours. The *blaOXA-51* gene was detected in all 123 (100%) isolates, confirming its intrinsic association with *A. baumannii* and demonstrating statistical significance ($p = 0.049$). This gene was present in 73.1% of blood isolates and 26.9% of endotracheal aspirate isolates.

CONCLUSION: The CarbAcineto NP test is a valuable rapid phenotypic assay for detecting carbapenemase activity in *A. baumannii*. The consistent presence of the *blaOXA-51* gene across all isolates underscores its utility as a reliable species-specific marker. Integrating these phenotypic and molecular diagnostic tools is crucial for accurate identification of CRAB, guiding effective antimicrobial stewardship, and implementing robust infection control strategies in tertiary care settings.

KEYWORDS: *Acinetobacter baumannii*, CarbAcineto NP test, *blaOXA-51* gene, carbapenem resistance, molecular diagnostics, phenotypic detection, species identification.

INTRODUCTION

Acinetobacter baumannii has emerged as a formidable nosocomial pathogen, particularly in intensive care units (ICUs), where it is a leading cause of severe invasive infections such as septicemia and ventilator-associated pneumonia [1]. The escalating global prevalence of carbapenem-resistant *A. baumannii* (CRAB) represents a critical public health concern, primarily driven by the production of carbapenemase enzymes that compromise the efficacy of last-resort

antibiotics [2, 6]. The timely and accurate identification of these resistant strains is paramount for guiding effective treatment strategies and preventing hospital outbreaks [7].

Traditional methods for detecting carbapenem resistance can be time-consuming, often delaying the initiation of appropriate antimicrobial therapy. To address this, rapid diagnostic tools have been developed. Among phenotypic assays, the CarbAcineto NP test has gained recognition for its speed and reliability in detecting carbapenemase activity [3, 8]. Concurrently, molecular techniques, such as Polymerase Chain Reaction (PCR), offer precise identification of resistance genes, providing insights into the genetic basis of carbapenem resistance and confirming species identity [4, 9].

One such molecular marker, the *blaOXA-51* gene, is intrinsically present in *A. baumannii* and serves as a species-specific identifier [5, 10]. Its consistent detection can confirm the presence of *A. baumannii* while its co-occurrence with carbapenemase activity, as detected by phenotypic tests, provides a comprehensive diagnostic picture. This paper aims to detail the findings from a study that utilized both the CarbAcineto NP test and *blaOXA-51* gene detection to characterize CRAB isolates from invasive clinical specimens, highlighting the synergistic value of these diagnostic approaches.

METHODOLOGY

A cross-sectional study was conducted at Index Medical College Hospital & Research Centre, Indore, involving 123 non-duplicate invasive *A. baumannii* isolates. These isolates were primarily obtained from blood and endotracheal aspirates. The study employed both phenotypic and molecular methods for the characterization of carbapenem resistance.

Phenotypic Detection of Carbapenemase Activity: CarbAcineto NP Test

Carbapenemase production was assessed using the CarbAcineto NP test. This assay is designed to detect carbapenem hydrolysis by observing a color change due to pH alteration. The test involves resuspending isolates in a hyperosmotic 5 M NaCl solution for bacterial lysis, followed by incubation with a phenol red solution containing zinc sulfate and imipenem. A rapid color change from red to yellow indicates carbapenemase activity. The sensitivity and specificity of the CarbAcineto NP test have been reported to be high for identifying carbapenemase-producing *Acinetobacter* spp. [3, 8].

Molecular Detection of *blaOXA-51* Gene

Molecular analysis was performed using PCR to detect the presence of specific carbapenemase genes. A primary focus was on the *blaOXA-51* gene, which is known to be intrinsically present in *A. baumannii* and serves as a reliable species-specific marker [5, 10]. The detection of this gene confirms the identity of the *Acinetobacter* species as *A. baumannii* and provides a baseline for understanding the genetic landscape of carbapenem resistance within the isolates. Results

Carbapenemase Production by CarbAcineto NP Test

Among the 123 *Acinetobacter baumannii* isolates tested, a significant majority, 91.1% (n=112), were identified as carbapenemase producers using the CarbAcineto NP test. The distribution of these carbapenemase-producing isolates varied by sample type: 74.1% (n=83) were isolated from blood samples, and 25.9% (n=29) were from endotracheal aspirates.

The remaining 8.9% (n=11) were non-carbapenemase producers, with 63.6% (n=7) from blood and 36.4% (n=4) from endotracheal aspirates. The phenotypic detection of carbapenemase production showed a statistically significant association with sample type ($p = 0.032$), indicating a notably higher detection rate in blood isolates compared to endotracheal aspirates.

Further analysis of the carbapenemase-producing isolates revealed variations in detection time:

- **Rapid Activity:** 14 isolates (11.3%) exhibited a rapid color change from red to yellow within 15 minutes, indicating strong carbapenemase activity.
- **Delayed Activity:** 98 isolates (79.6%) turned positive after 2 hours of incubation, suggesting a delayed but definitive enzymatic activity.

These findings underscore the high prevalence of carbapenemase-producing *A. baumannii* and suggest differences in enzyme expression levels among the isolates.

Detection of *blaOXA-51* Gene

Molecular analysis confirmed the presence of the *blaOXA-51* gene in all 123 (100%) of the *Acinetobacter baumannii* isolates. This universal detection of *blaOXA-51* confirms its intrinsic association with the species and its utility as a

species-specific marker. The statistical significance associated with its detection ($p = 0.049$) further reinforces its diagnostic utility. Specifically, the gene was detected in 73.1% of blood isolates and 26.9% of endotracheal aspirate isolates.

DISCUSSION

The findings from this study highlight the critical role of both phenotypic and molecular methods in the accurate and timely characterization of carbapenem-resistant *Acinetobacter baumannii*. The high rate of carbapenemase production detected by the CarbAcineto NP test (91.1%) underscores the significant challenge posed by CRAB in the studied tertiary care hospital [2, 6]. The observed variations in the speed of color change (15 minutes vs. 2 hours) suggest potential differences in the expression levels or types of carbapenemases present, which could have implications for the rapidity of phenotypic detection in clinical settings [8].

The consistent detection of the *blaOXA-51* gene in all 123 *A. baumannii* isolates is particularly noteworthy. This gene is widely recognized as an intrinsic chromosomal marker for *A. baumannii*, making its presence a reliable indicator for species identification [5, 10]. The statistical significance associated with its detection further reinforces its diagnostic utility. In a clinical microbiology laboratory, the ability to rapidly confirm *A. baumannii* identity alongside carbapenemase activity is invaluable for guiding infection control measures and initiating appropriate antimicrobial therapy [7, 9].

Integrating the CarbAcineto NP test with *blaOXA-51* gene detection offers a comprehensive diagnostic approach. The phenotypic test provides a quick screen for carbapenemase activity, while the molecular test confirms the species and the genetic basis of resistance. This dual approach can enhance the precision of diagnosis, especially in cases where phenotypic results might be ambiguous or delayed [4, 11]. The statistically significant association between carbapenemase production and sample type (blood vs. endotracheal aspirates) also provides important epidemiological insights, suggesting potential differences in the clinical presentation or acquisition routes of CRAB infections [12].

CONCLUSION

This study demonstrates the robust utility of combining the CarbAcineto NP test and *blaOXA-51* gene detection for the comprehensive characterization of carbapenem-resistant *Acinetobacter baumannii* isolates. The CarbAcineto NP test proved effective in rapidly identifying carbapenemase activity, while the *blaOXA-51* gene served as a consistent and statistically significant marker for *A. baumannii* species identification. The integration of these phenotypic and molecular diagnostic tools is essential for enhancing the accuracy and speed of CRAB detection, which is critical for informing antimicrobial stewardship programs, guiding targeted therapeutic interventions, and strengthening infection control practices in healthcare environments [1, 2, 6]. Continued surveillance using such integrated approaches will be vital in combating the ongoing threat of antimicrobial resistance posed by *A. baumannii* [7, 12].

REFERENCES

1. Wong D, Nielsen TB, Bonomo RA, Pantapalangkoor P, Luna B, Spellberg B. Clinical and Pathophysiological Overview of Acinetobacter Infections: a Century of Challenges. *Clin Microbiol Rev.* 2017 Jan;30(1):409–47.
2. Cisneros JM, Rodríguez-Baño J. Nosocomial bacteremia due to *Acinetobacter baumannii*: epidemiology, clinical features and treatment. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis.* 2002 Nov;8(11):687–93.
3. Dortet L, Poirel L, Errera C, Nordmann P. CarbAcineto NP test for rapid detection of carbapenemase-producing *Acinetobacter* spp. *Journal of clinical microbiology.* 2014 Jul 1;52(7):2359-64.
4. Paul DR, Singh DK. *Fundamentals of Molecular Diagnostics in Clinical Microbiology.* Dentomed Publication House; 2024. 151 p.
5. Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL. Identification of *Acinetobacter baumannii* by detection of the *blaOXA-51*-like carbapenemase gene intrinsic to this species. *Journal of clinical microbiology.* 2006 Aug 1;44(8):2974-6.
6. Falagas ME, Kopterides P. Risk factors for the isolation of multi-drug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*: a systematic review of the literature. *J Hosp Infect.* 2006 Sep;64(1):7–15.
7. Schinas G, Polyzou E, Spernovasilis N, Gogos C, Dimopoulos G, Akinosoglou K. Preventing Multidrug-Resistant Bacterial Transmission in the Intensive Care Unit with a Comprehensive Approach: A Policymaking Manual. *Antibiotics.* 2023 Jul 30;12(8):1255.
8. Dortet L, Poirel L, Errera C, Nordmann P. CarbAcineto NP test for rapid detection of carbapenemase-producing *Acinetobacter* spp. *Journal of clinical microbiology.* 2014 Jul 1;52(7):2359-64.
9. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, Amyes SG, Livermore DM. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. *international journal of antimicrobial agents.* 2006 Apr 1;27(4):351-3.
10. Amudhan SM, Sekar U, Arunagiri K, Sekar B. OXA beta-lactamase-mediated carbapenem resistance in *Acinetobacter baumannii*. *Indian journal of medical microbiology.* 2011 Jul 1;29(3):269.



11. Lin MF, Lan CY. Antimicrobial resistance in *Acinetobacter baumannii*: From bench to bedside. *World J Clin Cases WJCC*. 2014 Dec 16;2(12):787–814.
12. Hsu LY, Apisarnthanarak A, Khan E, Suwantararat N, Ghafur A, Tambyah PA. Carbapenem-Resistant *Acinetobacter baumannii* and *Enterobacteriaceae* in South and Southeast Asia. *Clin Microbiol Rev*. 2017 Jan;30(1):1–22.