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Original Article

Multiplex PCR for identification and β-lactam resistance gene detection in clinical isolates of *Acinetobacter baumannii*

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Abstract

Introduction: *Acinetobacter baumannii* (*A. baumannii*) is a major cause of hospital-acquired infections and frequently harbors antibiotic-resistance genes that complicate treatment. Rapid identification and resistance gene detection are essential for effective antibiotic use and infection control. This study developed a multiplex polymerase chain reaction (PCR) assay to identify *A. baumannii* and detect key β-lactam resistance genes for clinical isolates.

Methodology: The assay targeted the *recA* gene and the 16S-23S ribosomal RNA internal transcribed spacer region for *A. baumannii* identification. In addition, five β-lactamase genes (*bla*$_{\text{OXA-51-like}}$, *ampC*, *bla*$_{\text{CTX-M}}$, *bla*$_{\text{TEM}}$, and *bla*$_{\text{SHV}}$) were targeted. Antimicrobial susceptibility testing and phenotypic extended-spectrum beta-lactamase detection were performed to confirm resistance profiles.

Results: Of the 49 *Acinetobacter* isolates, 46 were identified as *A. baumannii* by multiplex PCR. All 46 isolates contained *ampC*, and 45 harbored *bla*$_{\text{OXA-51-like}}$. *bla*$_{\text{TEM}}$ was detected in 34 isolates, whereas *bla*$_{\text{CTX-M}}$ and *bla*$_{\text{SHV}}$ were absent. Phenotypic tests showed general agreement with the PCR results. High resistance rates were observed for multiple antibiotic classes, including carbapenems, cephalosporins, and aminoglycosides.

Conclusions: The multiplex PCR assay developed here provides a rapid and reliable method for *A. baumannii* identification and resistance gene detection, outperforming conventional methods in terms of speed and accuracy. The high resistance rates observed highlight the urgent need for effective diagnostic tools and infection control strategies to combat multidrug-resistant *A. baumannii*.

Key words: *Acinetobacter*; β-lactamase; multiplex-PCR; resistance; carbapenem.

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Introduction

Acinetobacter baumannii (*A. baumannii*) has emerged as a significant global health concern, particularly in hospital-acquired infections [1]. This Gram-negative bacterium has garnered attention not only because of its increasing prevalence but also because of its remarkable ability to acquire antibiotic resistance mechanisms [2]. Of particular concern is the organism's capacity to produce enzymes that hydrolyze novel antibiotics, including carbapenems, which are often considered drugs of last resort [3].

A. baumannii has emerged as a formidable nosocomial pathogen characterized by its remarkable ability to persist in hospital environments through desiccation resistance, biocide tolerance, and minimal nutritional requirements [4]. The global prevalence of carbapenem-resistant *A. baumannii* (CRAB) strains has risen dramatically, with some regions reporting resistance rates exceeding 50% [5]. Statistics from the United States indicate a 78% increase in hospital-onset *A. baumannii* infections between 2019 and 2020, whereas studies from Italy have documented alarming surges in CRAB colonization and infection rates, particularly in coronavirus disease 2019 (COVID-19) intensive care units [6]. These trends underscore the urgent need for rapid and accurate diagnostic tools for the identification of *A. baumannii* and the detection of β-lactam resistance genes in clinical isolates for timely and effective patient management, infection control, and antimicrobial stewardship in the face of this increasingly resistant pathogen.

In Vietnam, the prevalence of multidrug-resistant *A. baumannii* has increased, particularly in hospitals where the burden of healthcare-associated infections is high [7]. This study aimed to fill an important gap in our understanding of local resistance patterns and their

potential impact on public health, providing valuable insight into the regional relevance of *A. baumannii* resistance in Southern Vietnam.

The rapid spread of antibiotic resistance in *A. baumannii* has sparked debate within the scientific community regarding the most effective approaches for the identification and characterization of resistant strains. Although traditional microbiological methods, such as biochemical and antimicrobial susceptibility assays, remain the gold standards, they are time-consuming and may delay critical treatment decisions [8,9]. This has led to increased interest in molecular techniques, particularly multiplex polymerase chain reaction (PCR), which offers the potential for simultaneous bacterial identification and the detection of antibiotic-resistance genes [10,11].

However, there is an ongoing discussion about which genetic markers are most appropriate for these purposes. Some researchers have advocated the use of the conserved *recA* gene for *A. baumannii* identification, whereas others have focused on the species-specific 16S-23S ribosomal RNA (rRNA) internal transcribed spacer (ITS) region [12,13]. Similarly, the relative importance of different β-lactamase genes in conferring clinically relevant resistance remains a subject of investigation [2].

This study aimed to address these knowledge gaps by developing and validating a multiplex PCR assay for the rapid identification of *A. baumannii* and the detection of key β-lactam resistance genes. We focused on the $bla_{\text{OXA-51-like}}$ gene (associated with carbapenem resistance); *ampC* gene (linked to cephalosporin resistance); and three genes encoding extended-spectrum β-lactamases (ESBL): $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV}. This study provided a comprehensive molecular profile of clinical *A. baumannii* isolates, which may contribute to more rapid and informed antibiotic prescription practices, potentially improving patient outcomes and slowing the spread of antibiotic resistance.

Methodology

Study design and duration

This cross-sectional study was designed to examine the molecular characteristics and antibiotic resistance profiles of *Acinetobacter* spp. isolates obtained from respiratory samples. The study was conducted from January 2023 to December 2023, during which bacterial isolates were collected from Binh Duong General Hospital in Vietnam and subjected to molecular and phenotypic analysis.

Bacterial strains

A total of 49 *Acinetobacter* spp. isolates were included in this study. These isolates were primarily obtained from bronchial fluid samples collected from Binh Duong General Hospital, Vietnam. The isolates were previously identified as *Acinetobacter* spp. using standard biochemical tests conducted at the hospital's microbiology laboratory. For this study, the isolates were cultured in Tryptic Soy Broth (TSB; Merck KGaA, Darmstadt, Germany) and streaked onto MacConkey Agar (MCA; Merck KGaA, Darmstadt, Germany) to isolate colonies for subsequent analysis via colony PCR.

Sample size justification

The sample size of 49 isolates was based on the availability of *Acinetobacter* spp. strains from the hospital's collection during the study period. No formal statistical power calculation was performed, but the sample size was sufficient to allow for meaningful molecular analysis and the detection of antibiotic resistance patterns within the scope of the study. Given the variability in the prevalence of resistant strains in clinical settings, this sample size was deemed adequate for the intended molecular and phenotypic analyses.

Rapid detection of A. baumannii using multiplex PCR (RD-PCR)

Multiplex PCR was used to identify *Acinetobacter* spp. at the genus level, specifically *A. baumannii*. The conserved *recA* gene (425 bp) was targeted for genus identification, whereas the species-specific ITS region of the *16S-23S rRNA* gene (208 bp) was used to identify *A. baumannii*. The primers used in these PCR assays are detailed in Supplementary Table 1.

The use of *recA* as a genus marker was selected due to its highly conserved nature across *Acinetobacter* species, allowing for reliable genus-level identification [14]. The ITS region was selected for species-specific identification of *A. baumannii* because of its distinct variability, making it an effective marker for differentiating *A. baumannii* from other *Acinetobacter* species in clinical isolates [12].

The RD-PCR reaction mixture (25 µL total volume) consisted of: 2.5 µL PCR buffer (10X), 1.0 µL each of dNTPs (10 mM), 4.0 µL $MgSO_4$ (25 mM), 1.0 µL each of primers (10 µM), 0.1 µL *Taq* DNA polymerase (5.0 U/µL), 1.0 µL bacterial suspension (one colony suspended in 20 µL TSB medium), and nuclease-free water. PCR amplification was performed using a Labnet MultiGene™ OptiMax thermal cycler (Labnet International, Edison, USA) with the following

conditions: initial denaturation (94°C for 3 minutes); followed by 30 cycles of denaturation (94°C for 30 seconds), annealing (51°C for 30 seconds), and extension (72°C for 45 seconds); and final extension (72°C for 5 minutes). The PCR products were analyzed by electrophoresis on 1% agarose gels (Sigma-Aldrich, St. Louis, MO, USA) containing SafeView (ABM Inc., Richmond, BC, Canada). Gel visualization was performed using a Gel Documentation System WGD-30 (DAIHAN Scientific, Wonju-si, Gangwon-do, South Korea). *Bacillus subtilis* (*B. subtilis*) strain PY79 was used as the negative control.

Detection of β-lactamase genes in A. baumannii by multiplex PCR (B-PCR)

To detect β-lactamase genes in *A. baumannii*, multiplex PCR was performed to target the $bla_{\text{OXA-51-like}}$ gene along with primers for the ACI-5 and ACI-6 regions. The $bla_{\text{OXA-51-like}}$ gene was chosen as the primary marker for carbapenem resistance because it is widely recognized as a hallmark of carbapenem resistance in *A. baumannii* [15]. The presence of this gene strongly correlates with imipenem resistance, making it an ideal marker for assessing carbapenem resistance in clinical isolates [16]. The *ACI5-ACI6* primers were included to confirm species identity and provide a more robust diagnostic approach (Supplementary Table 1).

The B-PCR reaction mixture (25 µL total volume) contained: 2.5 µL PCR buffer (10X), 1.0 µL each of dNTPs (10 mM), 4.0 µL $MgSO_4$ (25 mM), 1.0 µL each of $bla_{\text{OXA-51-like}}$ primers and *ACI5-ACI6* primers (10 µM), 0.5 µL *Taq* DNA polymerase (5.0 U/µL), 1.0 µL bacterial suspension, and nuclease-free water. The PCR conditions were as follows: initial denaturation (94°C for 3 minutes); 30 cycles of denaturation (94°C for 1 minute), annealing (47°C for 1 minute), and extension (72°C for 1 minute); and final extension (72°C for 5 minutes). The PCR products were analyzed by electrophoresis on 1% agarose gels (Sigma-Aldrich, St. Louis, MO, USA). Nuclease-free water was used as a negative control.

Detection of ESBL genes in A. baumannii by multiplex PCR (E-PCR)

ESBL genes were detected using multiplex PCR targeting bla_{TEM}, bla_{SHV}, and $bla_{\text{CTX-M}}$ genes. The bla_{TEM}, bla_{SHV}, and $bla_{\text{CTX-M}}$ genes were selected as markers for ESBL production because they are the most commonly identified ESBL genes in *A. baumannii* [17]. The presence of these genes is associated with resistance to extended-spectrum cephalosporins, which are often used as first-line treatments for infections caused by *A. baumannii* [18].

The E-PCR reaction mixture (25 µL total volume) included: 2.5 µL PCR buffer (10X); 1.0 µL each of dNTPs (10 mM); 1.0 µL each of *TEM*, *SHV,* and *CTX-M* primers (10 µM); 0.2 µL *Taq* DNA polymerase (5.0 U/µL); 3.0 µL bacterial suspension; and nuclease-free water. The PCR conditions consisted of: initial denaturation (94°C for 3 minutes); 30 cycles of denaturation (94°C for 30 seconds), annealing (55°C for 1 minute), and extension (72°C for 1 minute); and final extension (72°C for 5 minutes). The PCR products were analyzed by electrophoresis on 1% agarose gels (Sigma-Aldrich, St. Louis, MO, USA). Nuclease-free water served as the negative control.

In vitro antibiotic susceptibility testing

A. baumannii colonies were suspended in 0.85% NaCl to achieve a 0.5 McFarland standard ($1–3 \times 10^8$ CFU/mL). This suspension was then diluted 100-fold to achieve a final concentration of 10^6 CFU/mL, which was spread evenly onto Mueller-Hinton Agar (MHA; Merck KGaA, Darmstadt, Germany).

Antibiotic susceptibility was determined using the disk diffusion method. The antibiotic discs used in this study included: imipenem (10 µg), cefepime (30 µg), cefotaxime (30 µg), amikacin (30 µg), colistin (10 µg), levofloxacin (5 µg), and doxycycline (30 µg). The plates were incubated at 37°C for 24 hours in ambient air. The inhibition zone diameters were measured using an electronic vernier caliper (Insize 1112-200, Suzhou, China). Blank paper disks served as negative controls. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) M100-Ed34 guidelines [19].

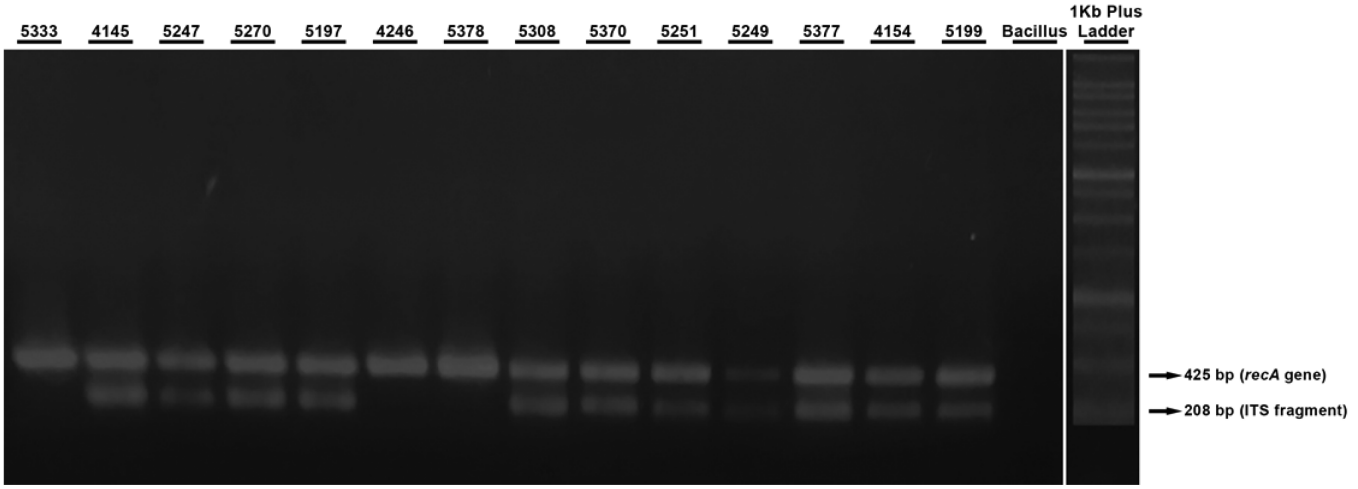
Rigorous quality control procedures were implemented following the CLSI guidelines to ensure the accuracy and reliability of the antibiotic susceptibility test results. The control strains, including *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213, were included in each batch of tests. These strains were tested concurrently with the study isolates to validate the performance of the antimicrobial agents and the integrity of the disk diffusion method.

The key quality control measures included:

(i) Inoculum density: The bacterial suspensions of control strains were 0.5 McFarland standard, ensuring uniform inoculum concentrations.

(ii) Antibiotic discs: Antibiotic discs were obtained from reputable suppliers and were stored according to

Figure 1. Multiplex PCR analyses of *Acinetobacter baumannii* and *Bacillus subtilis*.



Lane 1–14: *A. baumannii* clinical isolates; Lane 15: *B. subtilis* (negative control); Lane 16: DNA ladder.

the manufacturer's instructions to preserve their activity.

(iii) Incubation conditions: The plates were incubated at 37°C for 18–24 hours in ambient air, with the temperature monitored to ensure accurate testing conditions.

(iv) Interpretation of results: The inhibition zone diameters of control strains were compared against CLSI-established standards. Deviations from the accepted ranges prompted a review of the testing procedure and equipment calibration to ensure that the results were valid.

The accuracy of the antibiotic susceptibility testing was ensured by following these quality control protocols, and reliable data were obtained for the *A. baumannii* isolates.

Phenotypic detection of ESBL production using a combined disc test

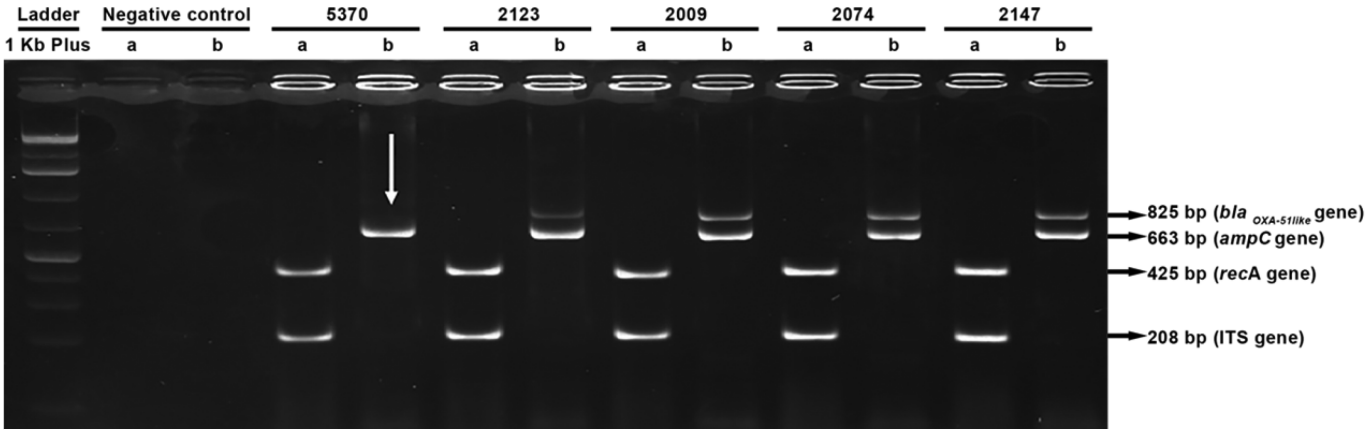
Phenotypic detection of ESBL production in *A. baumannii* isolates was performed using the combined disc test method. Antibiotic discs (Nam Khoa Biotek, Ho Chi Minh City, Vietnam) were used both alone and in combination (Supplementary Table 2). The results were evaluated according to the criteria for ESBL production based on the observed synergistic effects of the discs.

Results

Detection of A. baumannii using multiplex PCR

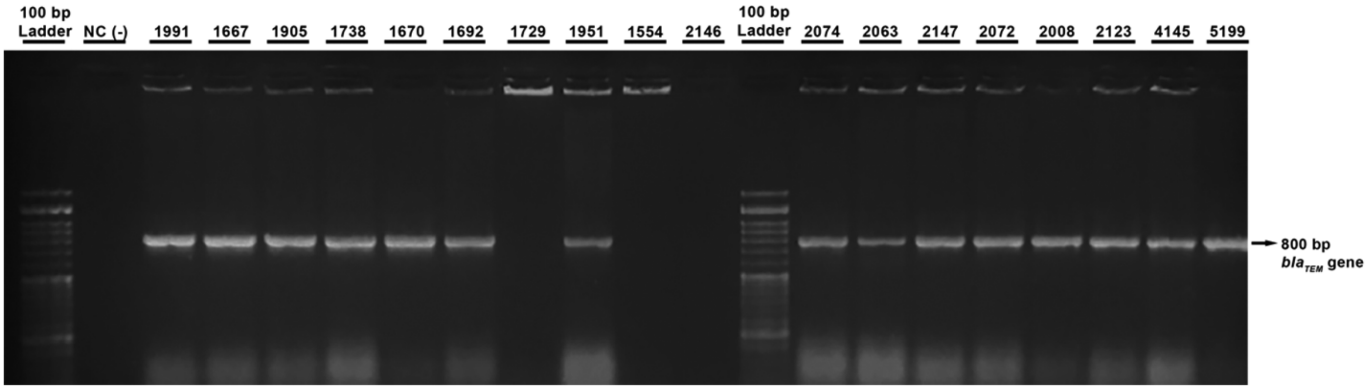
Of the 49 *Acinetobacter* spp. isolates subjected to multiplex PCR, 46 (93.9%) yielded two PCR products: ~425 bp (*recA* gene) and ~208 bp (*16S-23S rRNA* fragment), confirming their identity as *A. baumannii*. The remaining three isolates (5333, 4246, 5378)

Figure 2. Detection of ß-lactamase genes in *Acinetobacter baumannii* isolates.



(a) *recA* gene and *ITS* fragment amplification; (b) bla_{OXA-51} and *ampC* gene amplification. Sample 5370 contains only *ampC*.

Figure 3. PCR amplification of the bla$_{TEM}$ gene in *Acinetobacter baumannii* isolates.



NC (-): negative control.

produced only the *recA* gene product (425 bp), indicating that they belonged to the genus *Acinetobacter*, but not *A. baumannii* (Figure 1). These results corroborated the biochemical identification using the IVD NK-IDS 14 GNR commercial kit (Nam Khoa Biotech, Ho Chi Minh City, Vietnam). The colony PCR technique employed in this study expedited the diagnostic process by eliminating the need for DNA extraction.

Multiplex PCR analysis revealed that 45 out of 46 *A. baumannii* isolates (97.8%) harbored both the *bla*$_{OXA-51-like}$ (~825 bp) and *ampC* (~663 bp) genes, conferring resistance to carbapenems and cephalosporins, respectively. One isolate (5370) contained only *ampC*. The presence of *ampC* in all 46 isolates indicated potential resistance to cephalosporin antibiotics (Figure 2).

ESBL gene detection in A. baumannii

The *bla*$_{TEM}$ gene was detected in 34 of 46 *A. baumannii* isolates (73.9%). Notably, neither *bla*$_{CTX-M}$ nor *bla*$_{SHV}$ genes were identified in any isolate (Figure 3).

In vitro antibiotic susceptibility testing

Antibiotic susceptibility testing was performed on all 46 *A. baumannii* isolates using seven antibiotics that are commonly used for infection: imipenem, cefepime, cefotaxime, amikacin, colistin, levofloxacin, and doxycycline. Table 1, Figures 4 and 5 present the inhibition zone diameters of 10 representative samples (4145, 5199, 5308, 5249, 5370, 5247, 5270, 4154, 5197, and 5377).

Phenotypic detection of ESBL production

The cefotaxime-clavulanic acid (CEFOCLA) method identified two isolates (2146 and 1672) as ESBL-positive. The cefotaxime-sulbactam (CEFOSUL) method was unsuitable for ESBL phenotype identification because of the absence of inhibition zones in most isolates. Seven isolates (5377, 5197, 5249, 5199, 2146, 1692, 1672) were identified as ESBL-positive when MHA containing 200 µg/mL cloxacillin.

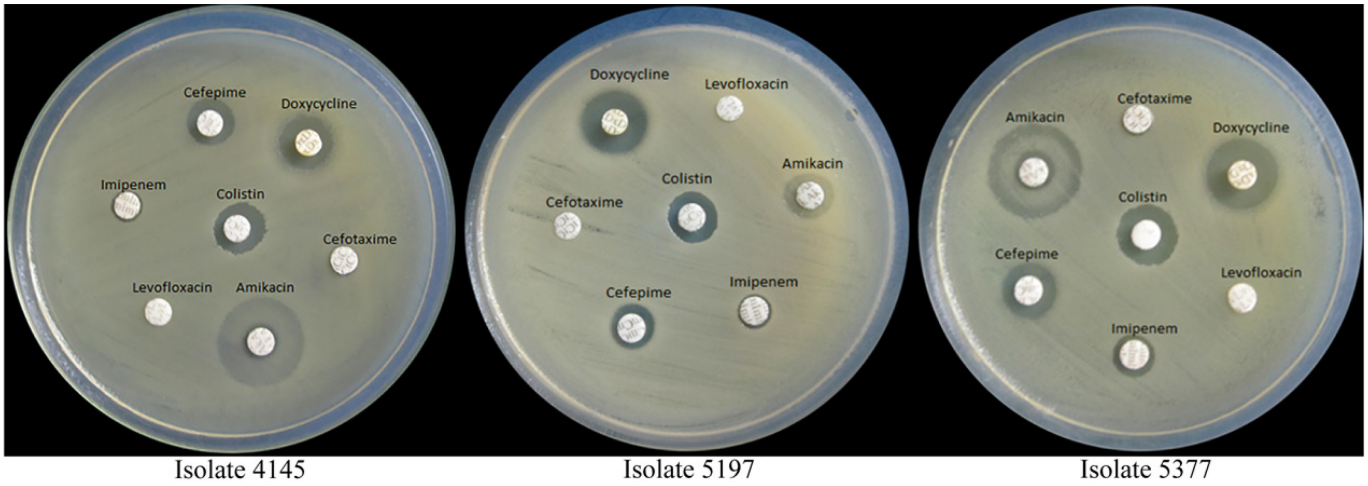
The cefotaxime-clavulanic acid-boronic acid (CEFO-CLA-BO) method detected ESBL production in 40 of 46 isolates (87%). Of these, 34 isolates

Table 1. Antibiotic susceptibility profiles of *Acinetobacter baumannii* isolates.

Isolates	Antibiotic						
	Im (mm) (IZD/REF)	Cm (mm) (IZD/REF)	Ct (mm) (IZD/REF)	Ak (mm) (IZD/REF)	Co (mm)* (IZD/REF)	Lv (mm) (IZD/REF)	Dx (mm) (IZD/REF)
4145	R (9/≤ 18)	R (12/≤ 14)	R (7/≤ 14)	R (7/ ≤14)	S (12/≥ 11)	R (7/≤ 13)	S (15/≥ 13)
5199	R (8/≤ 18)	R (8/≤ 14)	R (7/≤ 14)	R (7/ ≤14)	S (11/≥ 11)	R (7/≤ 13)	S (15/≥ 13)
5308	R (8/≤ 18)	R (8/≤ 14)	R (7/≤ 14)	R (7/ ≤14)	S (11/≥ 11)	R (7/≤ 13)	S (14/≥ 13)
5249	R (8/≤ 18)	R (8/≤ 14)	R (7/≤ 14)	R (14/ ≤14)	S (11/≥ 11)	R (7/≤ 13)	S (16/≥ 13)
5370	S (28/≥ 22)	S (22/≥ 18)	I (18/15–22)	S (23/ ≥17)	S (11/≥ 11)	R (12/≤ 13)	S (13/≥ 13)
5247	R (8/≤ 18)	R (12/≤ 14)	R (7/ ≤14)	R (7/ ≤14)	S (12/≥ 11)	R (7/≤ 13)	S (16/≥ 13)
5270	R (10/≤ 18)	R (10/≤ 14)	R (7/ ≤14)	I (15/15–16)	S (14/≥ 11)	R (7/≤ 13)	S (19/≥ 13)
4154	R (8/≤ 18)	R (11/≤ 14)	R (7/ ≤14)	R (7/≤ 14)	S (11/≥ 11)	R (7/≤ 13)	S (14/≥ 13)
5197	R (9/≤ 18)	R (10/≤ 14)	R (7/ ≤14)	R (7/≤ 14)	S (11/≥ 11)	R (7/≤ 13)	S (15/≥ 13)
5377	R (7/≤ 18)	R (9/≤ 14)	R (7/ ≤14)	R (7/≤ 14)	S (12/≥ 11)	R (7/≤ 13)	S (15/≥ 13)

IZD: inhibition zone diameter; REF: reference inhibition zone diameter for *A. baumannii* according to the Clinical and Laboratory Standards Institute (CLSI); Im: imipenem; Cm: cefepime; Ct: cefotaxime; Ak: amikacin; Co: colistin; Lv: levofloxacin; Dx: doxycycline; R: resistant; S: susceptible; I: intermediate. Colistin disc diffusion results are presented only for preliminary screening. According to updated CLSI guidelines, the determination of minimum inhibitory concentration (MIC) is recommended for definitive colistin susceptibility testing. Colistin results were not included in the final analysis because of poor disc diffusion.

Figure 4. Antibiotic susceptibility profiles of *Acinetobacter baumannii* isolates 4145, 5197, and 5377.



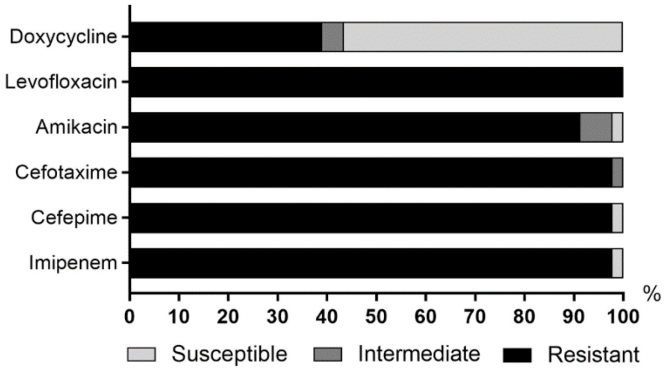
possessed the *bla*$_{TEM}$ gene, whereas none possessed the *bla*$_{SHV}$ or *bla*$_{CTX-M}$ genes. The presence of *ampC* in all isolates necessitated the use of boronic acid in the CEFO-CLA-BO method to inactivate the ampC β-lactamase enzyme, which is not inhibited by clavulanic acid or sulbactam.

Discussion

This study provides valuable insights into the antibiotic resistance patterns and genetic characteristics of *A. baumannii* isolates from Binh Duong General Hospital, Vietnam. Our findings highlight the alarming prevalence of multidrug-resistant *A. baumannii* in this region, which has profound implications for clinical practice and public health.

The results revealed high levels of resistance to multiple antibiotic classes among *A. baumannii* isolates. Notably, 97.8% (45/46) of the isolates exhibited resistance to imipenem, a carbapenem antibiotic that is often considered a last-resort treatment for multidrug-resistant infections. This high resistance rate was strongly correlated with the presence of the *bla*$_{OXA-51-like}$ gene, which was detected in all resistant isolates but absent in a single susceptible isolate (5370). This finding supports the critical role of *bla*$_{OXA-51-like}$ as a potential marker of carbapenem resistance in *A. baumannii* [20]. However, the precise mechanisms underlying carbapenem resistance, including possible alterations in porin or efflux pumps, require further investigation to comprehensively understand their resistance profiles of these species [21].

Figure 5. Antibiotic resistance rates among the 46 *Acinetobacter baumannii* clinical isolates.



A noteworthy discrepancy was observed between the genotypic and phenotypic resistance profiles. Although all isolates contained *ampC*, a high resistance rate was observed for cefepime (97.8%), a fourth-generation cephalosporin. However, a single susceptible isolate (5370) did not exhibit *ampC*-mediated resistance. This discrepancy may be attributed to variations in *ampC*, such as mutations or silent variants, which may affect gene expression or enzyme activity and thus influence phenotypic resistance [22,23]. This finding is consistent with previous studies, which showed that ampC-producing bacteria could remain susceptible to cefepime while displaying resistance to other cephalosporins [24]. Further sequencing of *ampC*, especially in the susceptible isolate (5370), could help clarify whether variations in the gene account for the observed susceptibility.

Similarly, high resistance to cefotaxime, a third-generation cephalosporin (100% resistance or intermediate resistance), was associated with the universal presence of *ampC*. This gene encodes *ampC* cephalosporinase, a class C β-lactamase that is known to confer resistance to many β-lactam antibiotics, which is consistent with the observed resistance profiles [25]. Resistance to other antibiotic classes was also noted: 97.8% resistance or intermediate resistance to amikacin (aminoglycoside), 100% resistance to levofloxacin

(fluoroquinolone), and 43.5% resistance or intermediate resistance to doxycycline (tetracycline).

The high resistance level observed in our study is consistent with findings from other regions in Southeast Asia, where *A. baumannii* has become a significant public health concern [26,27]. However, our study represents the first comprehensive report on the antibiotic resistance patterns of *A. baumannii* in Southern Vietnam, providing a valuable baseline for future comparative research. These results underscore the urgent need for increased surveillance, enhanced antimicrobial stewardship programs, and stricter infection control measures to mitigate the spread of multidrug-resistant *A. baumannii* [28].

Interestingly, molecular analysis revealed that 45 of the 46 *A. baumannii* isolates carried the $bla_{\text{OXA-51-like}}$ gene, confirming their species identity and potential for carbapenem resistance. Additionally, the presence of *ampC* in all cephalosporin-resistant isolates reinforces its role in β-lactam resistance. The detection of the bla_{TEM} gene in 73.9% (34/46) of isolates indicates a significant prevalence of ESBL production among these strains. This finding is consistent with previous reports of ESBL production by *A. baumannii* isolates from other regions [20].

However, our study revealed discrepancies in the phenotypic and genotypic identification of ESBL. Specifically, six isolates exhibited phenotypic ESBL production despite the absence of bla_{TEM}, bla_{SHV}, or $bla_{\text{CTX-M}}$ genes, indicating the presence of other ESBL-encoding genes or alternative mechanisms of resistance. This observation highlights the complexity of antibiotic resistance in *A. baumannii* and indicates the potential involvement of other uncharacterized ESBL genes [24]. These discrepancies emphasize the importance of complementing molecular diagnostic methods with phenotypic testing to capture the full spectrum of resistance mechanisms in clinical isolates.

The prevalence of ESBL genes differed between our isolates, diverging from findings in other regions. For example, studies conducted in Saudi Arabia and Iran reported different distributions of bla_{TEM}, bla_{SHV}, and $bla_{\text{CTX-M}}$ in *A. baumannii* isolates, reflecting geographical variability in resistance profiles [29]. These differences further underscore the importance of local surveillance programs tailored to specific regional resistance patterns, which are essential for developing effective treatment protocols and antimicrobial stewardship initiatives.

This study has limitations. The single-hospital origin of the samples and the relatively small sample size limit the generalizability of our findings to other regions of Vietnam or the broader population of *A. baumannii* isolates. The hospital-based nature of the study may introduce a selection bias because isolates from critically ill patients or patients with more severe infections are more likely to be included. Additionally, the relatively small sample size limits the statistical power of some of our analyses.

We recommend that future research expand the geographical scope by including multiple centers across different regions of Vietnam to provide a more comprehensive understanding of the resistance patterns of *A. baumannii*. Increasing the sample size would also allow more robust statistical analyses and improve the reliability of the findings. Additionally, longitudinal studies that track *A. baumannii* resistance over time could provide insights into the evolution of resistance mechanisms and the impact of antimicrobial stewardship programs [30].

Moreover, we suggest the inclusion of whole-genome sequencing in future studies to gain deeper insights into the genetic underpinnings of resistance. Whole-genome sequencing could enable the identification of novel resistance genes, reveal genomic variations contributing to resistance, and potentially track the spread of resistant clones across different settings [31]. These approaches will contribute to our understanding of the molecular mechanisms underlying the high resistance levels observed in *A. baumannii* and will help inform targeted interventions.

Overall, the findings of this study underscore the alarming prevalence of multidrug-resistant *A. baumannii* in Southern Vietnam, highlighting significant concerns regarding the treatment of infections caused by this pathogen. The observed discrepancies between phenotypic and genotypic resistance profiles point to the complexity of resistance mechanisms and emphasize the need for continued surveillance and research into the genetic basis of resistance. Comprehensive, multicenter studies and the integration of whole-genome sequencing will be critical for improving our understanding of *A. baumannii* resistance and informing effective public health strategies.

Conclusions

This study underscores the critical importance of rapid and accurate identification of *A. baumannii* and its associated antibiotic-resistance genes in clinical settings. Our research demonstrated the efficacy of molecular methods, particularly multiplex PCR techniques, in overcoming the limitations of traditional microbiological approaches. The successful

establishment of two multiplex PCR detection procedures for *A. baumannii*-specific genes and β-lactamase-producing genes, along with a simple PCR test for bla_{TEM}, offers significant advantages in terms of time, cost, and diagnostic accuracy. The high prevalence of multidrug-resistant *A. baumannii* observed in this study highlights the urgent need for enhanced infection control measures, judicious use of antibiotics, and the development of new therapeutic strategies. The molecular characterization of resistance mechanisms provides valuable insights for the development of rapid diagnostic tools and targeted therapies, which are crucial for informing public health policies and guiding clinical practice.

Hospital-acquired infections caused by *A. baumannii* continue to pose significant challenges to healthcare systems worldwide, and this study emphasizes the importance of continued surveillance and research into the evolution of antibiotic resistance. The integration of molecular techniques with traditional microbiological methods offers a powerful approach to address the growing threat of multidrug-resistant *A. baumannii* infections. Moving forward, expanding the geographical scope of surveillance, exploring novel therapeutic targets, and developing rapid, point-of-care diagnostic tools will be essential for facilitating timely and effective treatment strategies to combat this pressing public health concern.

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Conflict of interest

No conflict of interest is declared.

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Annex – Supplementary Items

Supplementary Table 1. Primers used for multiplex PCR detection of *Acinetobacter baumannii* and β-lactamase genes.

Primers	Sequence (5' - 3')	Size	Target
P-Ab-ITS-F	CATTATCACGGTAATTAGTG	208 bp	*Acinetobacter baumannii*
P-Ab-ITS-R	AGAGCACTGTGCACTTAAG		
P-rA1-F	CCTGAATCTTCTGGTAAAAC	425 bp	*Acinetobacter* spp.
P-rA2-r	GTTTCTGGGCTGCCAAACATTAC		
OXA-51-like-F	ATGAACATTAAAGCACTC	825 bp	$bla_{\text{OXA-51-like}}$ gene
OXA-51-like-R	CTATAAAATACCTAATTGTTC		
ACI5-F	ACTTACTTCAACTCGCGACG	663 bp	*amp*C gene
ACI6-R	TAAACACCACATATGTTCCG		
TEM-F	CATTTCCGTGTCGCCCTTATTC	800 bp	bla_{TEM} gene
TEM-R	CGTTCATCCATAGTTGCCTGAC		
CTX-M-F	TCTTCCAGAATAAGGAATCCC	909 bp	$bla_{\text{CTX-M}}$ gene
CTX-M-R	CCGTTTCCGCTATTACAAAC		
SHV-F	AGCCGCTTGAGCAAATTAAAC	713 bp	bla_{SHV} gene
SHV-R	ATCCCGCAGATAAATCACCAC		

Supplementary Table 2. Extended-spectrum beta-lactamase production in *Acinetobacter baumannii* isolates was determined using a combined disc test.

Method	Antibiotic	Testing medium	Interpretation of results
CEFOCLA	2 antibiotic discs: Cefotaxime (30 µg); cefotaxime/clavulanic acid (30/10 µg)	MHA	A ≥ 5 mm increase in inhibitory zone diameter (IZD) for a combination disc and a single antibiotic disc is regarded as ESBL positive (ESBL (+)), otherwise ESBL negative (ESBL (−))
CEFOSUL	2 antibiotic discs: Cefotaxime (30 µg); cefotaxime/sulbactam (30/10 µg)	MHA	See above
CLO-CEFOCLA	2 antibiotic discs: Cefotaxime (30 µg); cefotaxime/ clavulanic acid (30/10 µg)	MHA supplementary cloxacillin 200 µg/mL	See above
CEFO-CLA-BO	4 antibiotic discs: Cefotaxime (30 µg); Cefotaxime/clavulanic acid (30/10 µg); Cefotaxime/boronic acid (30/400 µg); Cefotaxime/clavulanic acid/boronic acid (30/10/400 µg)	MHA	A ≥ 5 mm increase in IZD for a cefotaxime/clavulanic acid/boronic acid disc versus a cefotaxime disc, or a ≥ 3 mm increase in IZD for a cefotaxime/clavulanic acid/boronic acid disc versus a cefotaxime/acid boronic disc indicates ESBL (+), otherwise ESBL (−)

CEFOCLA: cefotaxime (30 µg) and cefotaxime/clavulanic acid (30/10 µg); CEFOSUL: cefotaxime (30 µg) and cefotaxime/sulbactam (30/10 µg); CLO-CEFOCLA: cefotaxime (30 µg) and cefotaxime/clavulanic acid (30/10 µg), with cloxacillin to assess ampC β-lactamase activity; CEFO-CLA-BO, cefotaxime (30 µg), cefotaxime/clavulanic acid (30/10 µg), cefotaxime/boronic acid (30/400 µg), and cefotaxime/clavulanic acid/boronic acid (30/10/400 µg) for detecting ampC β-lactamase and serine carbapenemase activity; MHA, Mueller-Hinton agar, a culture medium used for antibiotic susceptibility testing.