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Comprehensive Analysis of Antibiotic Resistance Patterns in Clinical Isolates of *Acinetobacter baumannii*: A Cross-sectional Study in Karaj, Iran (2019)

Hamid Reza Farzaneh[1*], Saba Soufi[2], Ali Ahmadi[3], Nemat Karimi[2], Maryam Rahmani[4]

1. Aramis Laboratory, Rasht, Iran.
2. Department of Microbiology, Ka.C., Islamic Azad University, Karaj, Iran.
3. Department of Genetics, TeMS.C., Islamic Azad University, Tehran, Iran.
4. Department of Microbiology, Ro.C., Islamic Azad University, Roudehen, Iran.

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ABSTRACT

Background: *Acinetobacter baumannii* is an opportunistic gram-negative pathogen frequently associated with severe nosocomial infections and increasing multidrug resistance (MDR). This study aimed to compare the diagnostic performance of culture and PCR-based molecular methods targeting the $bla_{OXA-51-like}$ gene for the detection of *A. baumannii* and to evaluate the antibiotic resistance patterns of isolates obtained from respiratory samples.

Materials and Methods: In this descriptive cross-sectional study, 236 patients with respiratory illnesses who were admitted to tertiary care hospitals in Karaj, Iran, during 2019 were enrolled. Bronchoalveolar lavage (BAL) and induced sputum samples were collected. Bacterial culture and antimicrobial susceptibility testing (AST) were performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines, and polymerase chain reaction (PCR) assays were carried out using species-specific primers. Statistical analyses, including McNemar's test, were conducted using SPSS software, version 19.

Results: PCR detected *A. baumannii* in 75 samples (31.8%), while culture identified 70 samples (29.7%). PCR showed significantly higher sensitivity and specificity compared to culture ($P<0.05$). Antibiotic susceptibility testing revealed 100% resistance to ceftazidime, gentamicin, tobramycin, imipenem, ciprofloxacin, amikacin, cefepime, piperacillin, cefotaxime, ceftriaxone, piperacillin–tazobactam, doxycycline, tetracycline, and trimethoprim–sulfamethoxazole.

Conclusion: PCR demonstrated superior diagnostic accuracy compared to conventional culture for detecting *A. baumannii* in respiratory samples. The universal resistance observed underscores the urgent need for continuous surveillance, strict infection control measures, and the implementation of rapid molecular diagnostics to guide appropriate antimicrobial therapy in hospital settings.

*** Corresponding Author:**
Hamid Reza Farzaneh
Address: *Aramis Laboratory, Rasht, Iran.*
Phone: *+98 (13) 33220085*
E-mail: *aramislaboratory@gmail.com*

Introduction

Acinetobacter baumannii is a gram-negative, aerobic, non-fermentative, oxidase-negative coccobacillus. This opportunistic pathogen is among the leading causes of nosocomial infections and can be isolated from various clinical specimens, including blood, sputum, pleural fluid, and urine, particularly in patients with device-associated infections [1]. It is recognized as a primary pathogen in patients admitted to intensive care units (ICUs), where it can persist for prolonged periods in hospital environments, contributing significantly to healthcare-associated infections [2].

A. baumannii is part of the normal skin flora in healthy individuals and is frequently found on moist body sites. Additional reservoirs include both wet and dry hospital surfaces, as well as medical equipment [3]. The bacterium's remarkable ability to survive on dry surfaces for days or weeks facilitates its persistence in clinical settings. Mortality associated with *A. baumannii* infections is often linked to treatment failure, underscoring the importance of accurate identification. Precise identification of *Acinetobacter* species is critical for antimicrobial susceptibility prediction, effective treatment selection, confirmation of diagnosis, epidemiological investigations, and environmental biodiversity studies [4].

Traditional identification methods, such as isolation, direct microscopic examination, and bacterial culture are commonly used but are time-consuming and have inherent limitations. Molecular techniques, particularly polymerase chain reaction (PCR), offer rapid, sensitive, cost-effective, and straightforward detection by amplifying species-specific DNA sequences [5]. PCR also enables direct diagnosis from clinical specimens, bypassing some challenges associated with culturing environmental contaminants.

Respiratory infections caused by *A. baumannii* have shown a rising global trend [6]. The bacterium is frequently isolated from hospital environments and patients, especially those in ICUs who have undergone intubation or possess indwelling vascular catheters, as it preferentially colonizes aqueous environments [7].

In recent years, the management of *Acinetobacter* infections has been complicated by increasing antibiotic resistance, largely driven by antibiotic overuse. Many isolates exhibit multidrug resistance (MDR), defined as resistance to at least three antibiotic classes, such as third-generation cephalosporins, aminoglycosides, and fluoroquinolones [8]. Treatment options for these infections include colistin, amikacin, tigecycline, and novel glycolysis inhibitors; however, resistance against these agents has also been reported [1, 4, 9]. Furthermore, pan-drug-resistant (PDR) strains, resistant to nearly all available antimicrobials except colistin and polymyxin B, have emerged [10]. Extensive drug-resistant (XDR) strains, resistant to almost all antibiotics, pose significant treatment challenges [11]. These resistant isolates are associated with prolonged hospital stays, increased healthcare costs, and elevated mortality rates.

Since the introduction of broad-spectrum beta-lactams, such as carbapenems in 1985, these antibiotics have been widely used against MDR *Acinetobacter* infections [12]. Nonetheless, carbapenem resistance is escalating globally. Resistant isolates frequently demonstrate complete resistance to most antimicrobials and partial resistance to rifampin [6, 8, 11]. Mechanisms of carbapenem resistance include enzymatic degradation, efflux pump activation, target site modification, and changes in outer membrane permeability [13].

Given these concerns, this study aimed to achieve two main objectives aligned with the Ministry of Health and Medical Education's priorities. First, it sought to develop and validate a molecular PCR method for the specific detection of *A. baumannii* and to compare its diagnostic performance with routine methods approved by the Ministry. Since no comprehensive screening of *A. baumannii* in respiratory samples has been conducted in Iran, the findings could have significant implications for infection control, epidemiology, and early detection. Karaj, Iran's second-largest city, was selected as the study site. Second, this study aimed to determine the antibiotic resistance profiles of *A. baumannii* isolates from Karaj. The results are expected to provide essential data to guide clinicians, laboratory personnel, and researchers in managing this pathogen.

Materials and Methods

This descriptive cross-sectional study was conducted on 236 patients with respiratory symptoms referred to tertiary care hospitals in Karaj, Iran, during 2019. Patients with recent antibiotic use (within 14 days), underlying chronic lung diseases unrelated to infection, or insufficient sample volume were excluded. Both bronchoalveolar lavage (BAL) and induced sputum samples were collected according to clinical indications: BAL was performed mainly on ICU or immunocompromised patients with severe pneumonia, while induced sputum was obtained from other patients.

Samples were transported on ice to the microbiology laboratory. Each specimen was divided into two aliquots: One for culture and antimicrobial susceptibility testing (AST) and one for PCR analysis. Bacterial culture was performed on MacConkey agar and blood agar, followed by biochemical tests (triple sugar iron (TSI) agar, citrate utilization, sulfide indole motility (SIM), methyl red/Voges-Proskauer (MR/VP), and urease). AST was performed using the Kirby–Bauer disk diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) (2019) guidelines [14]. Quality control strains (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) were included.

For PCR, DNA was extracted using a commercial kit (Qiagen, Germany). Species-specific primers targeting the $bla_{OXA\text{-}51\text{-}like}$ gene were designed and validated. PCR products were visualized on 1% agarose gel electrophoresis, and band sizes were compared with a 100 bp DNA ladder. Positive identification was confirmed when the expected 346 bp band was observed.

All procedures were approved by the Ethics Committee of Shahrekord University of Medical Sciences and written informed consent was obtained from all participants.

Results

In this study, a total of 236 samples, including BAL and induced sputum, were collected from patients with respiratory symptoms who were referred to specialized and subspecialized hospitals in Karaj. Sample collection was performed via fiberoptic bronchoscopy in cooperation with the patients. Demographic data of the 236 samples are presented in Table 1.

The microbial culture method yielded positive results in 29.7% of cases and negative results in 70.3% of patients. Additionally, the PCR method detected *A. baumannii* in 31.8% of samples, with negative results in 68.2%. Comparison of the PCR and microbial culture results using the non-parametric McNemar test revealed a statistically significant difference between the two methods ($P<0.05$).

Computational analysis of the primers was conducted using BLAST software on the NCBI website. This analysis confirmed 100% homology of both primers with the target genome, indicating their specificity for different strains of *A. baumannii*. Primer analysis also demonstrated minimal non-specific binding, such as primer-dimer formation. For non-specific amplification to occur, a sequence similar to the genome must bind to

Table 1. Demographic information of the patients

Variables	Category	No. (%)
Gender	Female	83(35.2)
	Male	153(64.8)
Age (y)	<30	42(16.4)
	31–40	36(14.1)
	41–50	54(21.1)
	51–60	61(23.8)
	≥61	63(24.6)
Non-respiratory comorbidities	Diabetes	45(17.6)
	Hyperlipidemia	25(9.8)
	Hypertension	27(10.6)
	Combination of comorbidities	30(11.7)
	Other comorbidities	26(10.1)
	No comorbidity	103(40.2)

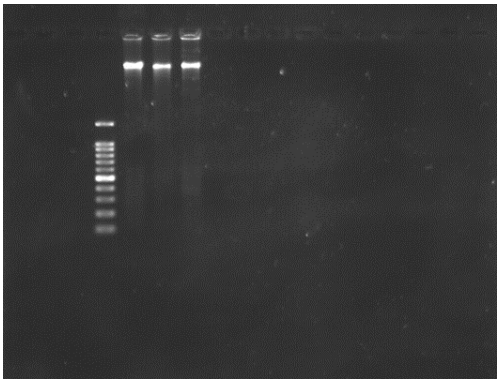


Figure 1. Comprehensive genomic analysis of extracted *A. baumanni*i strains

Table 2. PCR optimization results

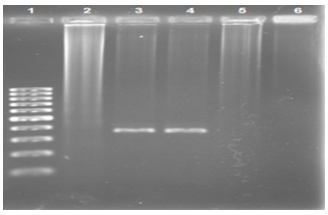
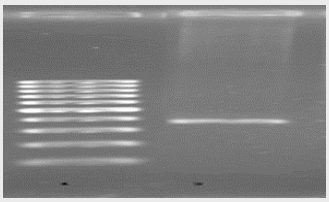
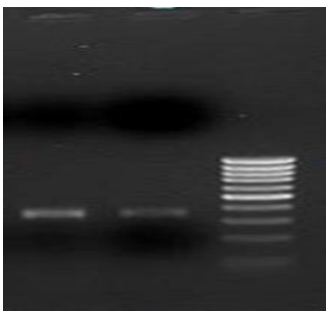
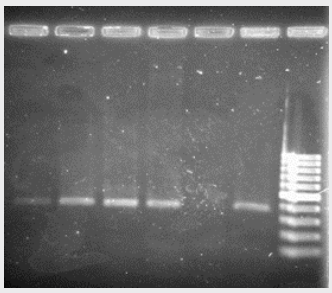
Test Description	Figures	Notes/Observations
Optimization of $MgCl_2$ concentration		Mg^{2+} concentrations tested: 0.5–2 mM. Optimal bands were observed at 0.8 and 1 mM.
Optimization of DNA sample concentration		A clear 346 bp PCR product was observed on a 1% agarose gel
Determination of required DNA volume		Lane 1: 100 bp ladder; Lane 2: 2.5 µL PCR product; Lane 3: 5 µL PCR product.
PCR results on BAL and sputum samples		After dilution, 2.5 µL was used. Specific amplification of the *A. baumannii* genome was observed.

Table 3. Antibiotic resistance pattern of *A. baumannii*

Antibiotic (Disc Content)	S (mm)	I (mm)	R (mm)	No. of Resistant Isolates (%)
Ceftazidime (30 µg)	≥18	15–17	≤14	70(100%)
Gentamicin (10 µg)	≥15	13–14	≤12	—
Tobramycin (10 µg)	≥15	13–14	≤12	—
Imipenem (10 µg)	≥16	14–15	≤13	—
Ciprofloxacin (5 µg)	≥21	16–20	≤15	—
Amikacin (30 µg)	≥17	15–16	≤14	70(100%)
Cefepime (30 µg)	≥18	15–17	≤14	—
Piperacillin (100 µg)	≥21	18–20	≤17	—
Cefotaxime (30 µg)	≥23	15–22	≤14	—
Ceftriaxone (30 µg)	≥21	14–20	≤13	—
Piperacillin–tazobactam	≥18	—	≤17	—
Doxycycline (30 µg)	≥15	12–14	≤11	—
Tetracycline (30 µg)	≥13	10–12	≤9	—
Trimethoprim–sulfamethoxazole	≥16	11–15	≤10	—

both primers; if similarity is present only in one primer, the formation of non-specific bands is unlikely.

The primers employed in this study, consistent with those used in previous research, were capable of detecting all tested strains, suggesting that this sequence is reliable for the identification of *A. baumannii* in clinical samples. Following gel electrophoresis and ethidium bromide staining, DNA bands corresponding to *A. baumannii* were visualized under UV light in wells containing the original DNA samples (Figure 1).

A summary of the optimized concentrations, PCR conditions, and DNA quantification results is presented in Table 2.

A. baumannii exhibited resistance to all antibiotics tested under laboratory conditions. The detailed results are presented in Table 3.

The antimicrobial susceptibility assessment indicated that *A. baumannii* was resistant to all antibiotics tested under laboratory conditions.

Discussion

The non-parametric McNemar test demonstrated that the sensitivity of the PCR method for detecting *A. baumannii* was higher than that of culture and routine bacteriological methods. Furthermore, the specificity of PCR in diagnosing *A. baumannii* was also superior compared to culture. These findings indicate a significant correlation between PCR and microbial culture methods in the identification of *A. baumannii* ($P<0.05$). This suggests that PCR is a more precise technique for accurately diagnosing patients infected with *A. baumannii* than conventional culture methods. Specifically, PCR detected 75 positive cases (31.8%) and 161 negative cases (68.2%), whereas microbial culture identified 70 positive cases (29.7%) and 166 negative cases (70.3%). Therefore, PCR can be considered a reliable alternative to culture and routine laboratory techniques for detecting *A. baumannii* in lavage and sputum samples.

Similar studies support these findings. For example, a 2011 study reported that among 60 samples from hospitalized pneumonia patients, 22 samples (36.7%) were positive for *A. baumannii* gene detection (API ID32GN), whereas culture was positive in only 6 patients (10%). In the mentioned study, PCR exhibited 100% sensitivity

and 70.4% specificity (with 38 negative cases), consistent with our current results. Geber et al. demonstrated that PCR could detect *A. baumannii* at concentrations as low as 10–20 CFU/mL. Positive PCR results were obtained 5 to 7.8 hours before a positive BACTEC™ signal. Using PCR, 11 of 18 respiratory samples were detected with an average lead time of 10.7 hours prior to the positive culture signal, aligning well with our findings [15].

Moreover, Farajnia et al. screened the PER1, PER2, and PER3 genes of *A. baumannii*, which encode extended-spectrum beta-lactamases (ESBLs), and reported that 51% of isolates from ICU patients were positive for the *PER1* gene by PCR [16]. These findings, alongside the current study, suggest that PCR demonstrates higher sensitivity and specificity than routine bacterial culture in detecting *A. baumannii* strains. The sensitivity of a diagnostic test indicates its ability to correctly identify diseased individuals, meaning a test with high sensitivity is reliable for ruling out disease when results are negative. Specificity reflects the ability to correctly identify healthy individuals, i.e. the proportion of true negatives. The present results showed that *A. baumannii* isolates exhibited resistance to all antibiotics tested in the laboratory, including ceftazidime, gentamicin, neomycin, imipenem, ciprofloxacin, amikacin, cefepime, piperacillin, doxycycline, tetracycline, and trimethoprim-sulfamethoxazole. Moghadam et al. reported resistance of *A. baumannii* isolates from sputum, wound secretions, and body fluids to aztreonam, ceftazidime, ciprofloxacin, piperacillin, and cefotaxime, consistent with our findings [17]. Farahani et al. reported that among 70 samples, 54% of *A. baumannii* isolates exhibited resistance to three or more antibiotics, and 32% were resistant to at least two antibiotics. Cefepime and ceftazidime were the most resistant antibiotics, whereas piperacillin-tazobactam, meropenem, and tobramycin showed higher sensitivity [18]. These results are partially inconsistent with the present study.

Overall, this study indicates a high prevalence of *A. baumannii* in the respiratory secretions of patients. Given the alignment of these findings with previous studies and considering *A. baumannii*'s ability to survive on hospital surfaces and medical equipment, identifying strains that produce various beta-lactamase enzymes is crucial for controlling and preventing the spread of resistant strains in healthcare settings.

Conclusion

PCR is a highly sensitive and specific method for the detection of *A. baumannii* in respiratory samples, outperforming traditional culture techniques. The high prevalence of MDR *A. baumannii* strains observed underscores the critical need for continuous surveillance and the implementation of effective infection control measures in hospital settings. Due to the extensive antibiotic resistance profile detected among isolates, timely and accurate identification of *A. baumannii* is essential to guide appropriate antimicrobial therapy and prevent the spread of resistant strains. These findings highlight the importance of incorporating molecular diagnostic tools alongside conventional methods to improve patient outcomes and limit healthcare-associated infections caused by *A. baumannii*.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of Shahrekord University of Medical Sciences, Shahrekord, Iran, and written informed consent was obtained from all participants.

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Authors contribution's

Study design and supervision: Hamid Reza Farzaneh and Nemat Karimi; Data collection, data, and writing the original draft: Hamid Reza Farzaneh and Saba Soufi; Review and editing: Ali Ahmadi and Maryam Rahmani.

Conflict of interest

The authors declared no conflict of interest.

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