RESEARCH PAPER

Molecular Identification of *Acinetobacter baumannii* from Burn Wound Infections Using blaOXA-51-like Gene PCR: A Comparative Study from a Tertiary Care Center in Odisha, India

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Abstract

Background: The emergence of *Acinetobacter baumannii* as a dominant nosocomial pathogen necessitates rapid diagnostic and molecular typing approaches, especially in burn units where vulnerable patients are at heightened risk of clonal dissemination and outbreak propagation.

Methods: During a six-month surveillance period, a total of 70 non-duplicate *Acinetobacter baumannii* isolates were recovered from burn wound swab specimens of hospitalized patients at a tertiary care burn center in Odisha. Preliminary identification was performed using conventional microbiological methods and subsequently confirmed by polymerase chain reaction (PCR) targeting the intrinsic *bla_{OXA-51-like}* gene.

Results :PCR analysis exhibited a high concordance rate of 95% with conventional phenotypic identification methods for the detection of *Acinetobacter baumannii* isolates. Out of the 70 isolates analyzed, 66 isolates showed agreement between both methods, while 4 isolates demonstrated discordance. This finding underscores the reliability of conventional methods, while also highlighting the superior specificity of PCR-based confirmation. Overall, PCR amplification of the intrinsic *bla*_{OXA-51-like} gene proved to be a robust and dependable method for confirming *Acinetobacter baumannii*.

Conclusion: PCR amplification of the bla_{OXA} gene provides a rapid and reliable approach for the identification of *Acinetobacter baumannii*. Implementation of this cost-effective molecular technique in routine diagnostics can facilitate early detection and improved infection control.

Keywords:*Acinetobacter baumannii*, Burn wound infections, Nosocomial infections, Molecular identification, bla_{OXA-51-like} gene.

How to cite this article: Rout S, Kumar R, Sahu KK. Molecular Identification of Acinetobacter baumannii from Burn Wound Infections Using blaOXA-51-like Gene PCR: A Comparative Study from a Tertiary Care Center in Odisha, India. Int J Drug Deliv Technol. 2026;16(54s): 402-407. DOI: 10.25258/ijddt.16.54s.35

Introduction

The genus *Acinetobacter* was first identified in the early twentieth century and comprises Gram-negative, strictly aerobic, non-fermentative, non-motile coccobacilli that are catalase-positive and oxidase-negative, with a genomic G+C content ranging between 37% and 49%. Clinically important species include *Acinetobacter baumannii*, *Acinetobacter calcoaceticus*, *Acinetobacter pittii*, and *Acinetobacter nosocomial is*, which are phylogenetically closely related and exhibit overlapping phenotypic features, making accurate differentiation by conventional biochemical techniques challenging [1][2]. Among these, *A. baumannii* is the species most frequently recovered from hospital environments and is widely regarded as a significant opportunistic pathogen. Other species such as *Acinetobacter pittii*, *Acinetobacter nosocomial is*, *Acinetobacter haemolyticus*, and *Acinetobacter johnsonii* are encountered less commonly. Members of this genus are ubiquitous in nature, being isolated from soil, water, food sources, and various hospital environments, including medical devices and equipment. Colonization in humans is not uncommon, particularly involving the skin and oropharynx, and is more frequently observed in patients subjected to invasive interventions such as tracheostomy [3]. Infections due to *Acinetobacter baumannii* are predominantly associated with intensive care settings and are more common in vulnerable populations, including immunocompromised individuals, elderly patients with comorbidities such as malignancies or extensive burn injuries, and those exposed to invasive procedures or prolonged courses of broad-spectrum antibiotics. In burn patients, infection with *A. baumannii* can delay wound healing and contribute to increased morbidity [4]. Accurate identification of *Acinetobacter baumannii* is crucial for effective clinical management and implementation of infection control measures. Conventional identification relies on colony morphology, Gram staining, and a

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range of biochemical tests, including oxidase and catalase activity, oxidative–fermentative reactions, citrate utilization, methyl red, Voges–Proskauer tests, motility assessment, and growth at different temperatures. However, these approaches often lack sufficient specificity because of the close phenotypic resemblance among related species [5][6]. Advances in molecular diagnostics have significantly enhanced the accuracy of identification. While techniques such as pulsed-field gel electrophoresis (PFGE), ribotyping, and plasmid profiling provide high discriminatory capability, their routine application is limited by technical complexity and cost. In contrast, polymerase chain reaction (PCR) targeting the intrinsic *bla*_{OXA-51-like} gene—naturally present on the chromosome of *A. baumannii*—is widely regarded as a dependable and practical method for species-level confirmation [7]. In the present study, isolates of *Acinetobacter baumannii* were initially identified using conventional phenotypic and biochemical methods, followed by molecular confirmation through PCR amplification of the *bla*_{OXA-51-like} gene. The study aimed to compare conventional and molecular approaches for accurate identification of *A. baumannii* in burn unit isolates [10]

Material and Methods

Phenotypic and genotypic identification of bacteria

This study was conducted on *Acinetobacter baumannii* isolates obtained from burn wound swab samples of patients admitted to a specialized burn center in Odisha, India. Initial identification was performed using standard microbiological and biochemical methods (Table 1). The isolates were collected from patients aged 2 to 75 years who had been hospitalized for at least one week.

Table 1. Phenotypic and biochemical characteristics used for identification of *Acinetobacter baumannii* isolates

Biochemical/Phenotypic Test	Observed Characteristics
Colony morphology on Blood Agar and MacConkey Agar	On blood agar: smooth, opaque to matte colonies without hemolysis or pigmentation. On MacConkey agar: purple to mucoid colonies.
Microscopic morphology (Gram staining)	Short, plump Gram-negative coccobacilli with a diplococcal appearance.
Catalase and Oxidase tests	Catalase positive; oxidase negative.
Triple Sugar Iron (TSI) test	Alkaline/alkaline (K/K) reaction without gas or hydrogen sulfide production.
Oxidative–Fermentative (OF) test	Oxidative utilization of glucose under aerobic conditions.
SIM test (Sulfur, Indole, Motility)	Non-motile; negative for indole and hydrogen sulfide production.
Growth temperature test	Growth observed at both 37 °C and 44 °C.

Molecular detection of *A. baumannii* isolates

Molecular confirmation of *Acinetobacter baumannii* isolates was performed using polymerase chain reaction (PCR) targeting the intrinsic *bla_{OXA-51}* gene. Genomic DNA from clinical isolates and reference strains was extracted using the boiling lysis method. *Acinetobacter baumannii* ATCC 19606 served as the positive control, while *Pseudomonas aeruginosa* ATCC 27853 was used as the negative control. For DNA extraction, 5–7 well-isolated colonies grown on nutrient agar were aseptically transferred into sterile 1.5 mL microcentrifuge tubes containing 350 µL of sterile deionized water. The bacterial suspension was subjected to thermal lysis by incubation in a boiling water bath for 10 minutes. After lysis, the tubes were gently vortexed and centrifuged at 12,000 rpm for 10 minutes at 4°C. The supernatant containing genomic DNA was carefully collected into fresh sterile tubes. The extracted DNA was either used immediately for PCR or stored at −20°C until further use, provided it met quality and concentration requirements. PCR amplification was carried out in a total reaction volume of 25 µL. The reaction mixture consisted of 11 µL of DNase/RNase-free water, 8 µL of 2× PCR Master Mix (containing 1.5 mM $MgCl_2$), 0.5 µL each of forward and reverse primers specific for *bla_{OXA-51}*, and 5 µL of template DNA. The primer sequences used are listed in Table II. Amplification was performed in a thermal cycler with an initial denaturation step at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute. A final extension step was carried out at 72°C for 10 minutes to ensure complete synthesis of PCR products. The amplified products were analyzed by agarose gel electrophoresis. A volume of 10 µL of each PCR product was loaded onto a 1% agarose gel prepared in TBE buffer (40 mM Tris, 20 mM boric acid, and 1 mM EDTA; pH 8.3) containing 0.5 µg/mL DNA SafeStain. Electrophoresis was conducted at 80 V for approximately 45 minutes. The gels were then visualized under ultraviolet light using a gel documentation system, and images were captured for record-keeping. Samples showing a distinct band at the expected amplicon size of the *bla_{OXA-51}* gene were considered positive for *A. baumannii*. To further validate the results, selected PCR products were subjected to sequencing. The obtained sequences were analyzed using Chromas and compared for similarity using BLAST tools available through the National Center for Biotechnology Information.

Table 2. Primer sequences used for amplification of the bla_{OXA-51} gene by PCR

Target Gene	**Primer Direction**	**Primer Sequence (5′–3′)**	**Amplicon Size (bp)**	**Reference**
bla_{OXA-51}	Forward	TAATGCTTTGATCGGCCTTG	353	Woodford et al., 2006 [21]
bla_{OXA-51}	Reverse	TGGATTGCACTTCATCTTGG	353	Woodford et al., 2006 [21]

Results

During a six-month study period, a total of 70 clinical isolates, preliminarily identified as *Acinetobacter baumannii* based on phenotypic and biochemical characteristics, were collected from a burn hospital in Odisha, India. Isolation and identification procedures were conducted in the diagnostic microbiology laboratory of a general hospital. The phenotypic and biochemical methods used for identification are summarized in Table 2. To confirm the identity of the isolates at the molecular level, polymerase chain reaction (PCR) targeting the *bla_{OXA-51}* gene was performed. Out of the 70 isolates, 66 (95%) were found to harbor the target gene, thereby confirming their identification as *A. baumannii*. The amplification of the *bla_{OXA-51}* gene yielded a product size of 353 base pairs, as illustrated in Figure 1. For further validation, one representative PCR product was subjected to DNA sequencing to confirm the molecular identity of the organism.

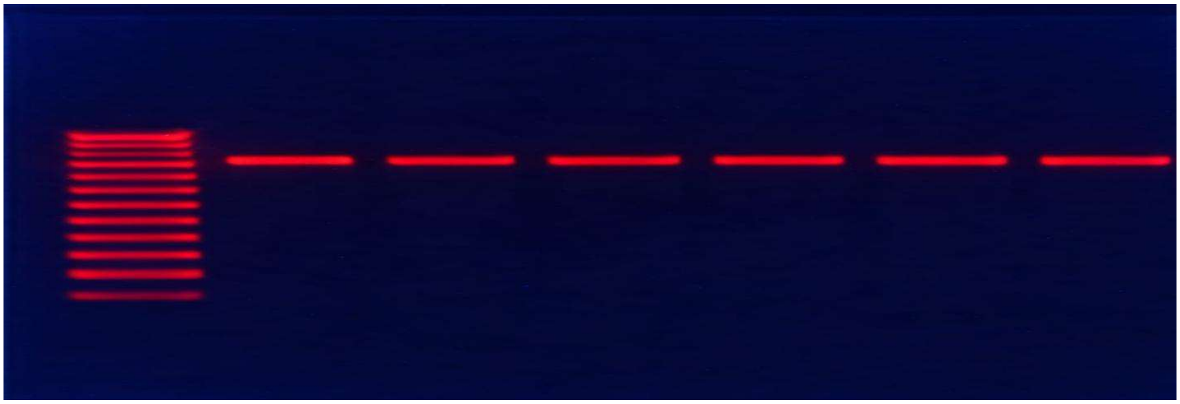

Figure 1. Agarose gel electrophoresis of the amplified PCR products confirmed the presence of the *bla*_{OXA-51} gene, producing the expected 353 bp amplicon in selected *Acinetobacter baumannii* isolates.

In the electrophoretic analysis, Lane M represented the 100 bp DNA ladder, while Lanes 1–6 showed clear bands at 353 bp, confirming successful amplification of the target gene. Pseudomonas aeruginosa ATCC 27853 was included as a negative control and showed no amplification, thereby validating the specificity of the PCR assay.

Discussion

The rising incidence of *Acinetobacter baumannii* infections in healthcare settings has become a major global public health issue, particularly in resource-limited countries. This opportunistic organism is now recognized as a prominent cause of hospital-acquired infections, largely owing to its ability to persist in the clinical environment and its extensive resistance to multiple classes of antimicrobial agents. The emergence and spread of multidrug-resistant (MDR) strains have significantly complicated treatment options and infection control measures, leading to increased morbidity and mortality among hospitalized patients. Evidence from various studies indicates that infections caused by MDR *A. baumannii* are associated with a considerable proportion of deaths related to nosocomial infections [11].

A key difficulty in assessing the actual burden of *A. baumannii* infections is differentiating true infection from mere colonization. The presence of the organism in clinical samples does not always imply active disease, as it may exist as a colonizer without causing symptoms. However, colonization with MDR strains is considered an important risk factor for the development of subsequent invasive infections, especially in high-risk and immunocompromised patient groups [12].

In India, similar to other regions worldwide, *A. baumannii* has become a prominent cause of hospital-acquired infections. High-risk groups include immunocompromised individuals, patients with underlying chronic diseases such as cystic fibrosis, and especially burn patients. Burn units and intensive care units (ICUs) are considered high-risk environments due to the frequent use of invasive procedures, prolonged hospitalization, and extensive antibiotic exposure. In recent years, *A. baumannii* has emerged as one of the predominant pathogens in these wards, with reported mortality rates among ICU patients ranging widely and, in some cases, reaching alarming levels [13][14].

Earlier studies from Odisha have documented notable isolation rates of *Acinetobacter baumannii* from patients in burn units and intensive care settings. In the present investigation, a total of 66 isolates were obtained over a six-month period from a tertiary burn care hospital, reflecting a considerable infection burden within a relatively limited duration. Such a high occurrence suggests the possibility of intra-hospital spread, as well as transmission between referring healthcare facilities. Since the study site serves as a major burn referral center and receives patients and samples from multiple institutions, the likelihood of inter-hospital dissemination cannot be ruled out. These observations emphasize the necessity for strict implementation of infection prevention and control practices, particularly in high-risk clinical areas [15].

Conventionally, identification of *Acinetobacter* species in routine clinical laboratories has been based on phenotypic and biochemical characteristics, including tests for glucose oxidation, gelatin hydrolysis, hemolysis, utilization of different carbon sources, and growth at varying temperatures. However, these methods may lack sufficient accuracy due to phenotypic similarities among closely related species, which can result in misidentification. In contrast, molecular diagnostic techniques provide greater sensitivity and specificity, along with faster results, thereby improving the reliability of species-level identification [16]. Although numerous studies in Iran have focused on antimicrobial resistance patterns of clinical *A. baumannii* isolates, data regarding their genetic diversity and clonal distribution remain limited. Molecular typing techniques provide valuable epidemiological insights by identifying transmission routes and determining whether infections are caused by clonal spread or unrelated strains [17]. *Acinetobacter* species are commonly recovered from moist areas of the skin and mucosal surfaces, including the respiratory and gastrointestinal tracts of hospitalized patients. Environmental contamination plays a crucial role in their transmission. The organism has been isolated from various hospital surfaces and medical devices such as ventilator circuits, respiratory equipment, blood pressure monitors, gloves, mattresses, and other frequently touched objects. Evidence from international studies suggests that the majority of *A. baumannii* infections are hospital-acquired, with only a small proportion originating from community settings. In many outbreaks, colonized or infected patients serve as the primary reservoir for transmission [17]. Effective prevention of *A. baumannii* outbreaks, particularly in burn units, requires strict adherence to infection control practices, including hand hygiene, environmental decontamination, antimicrobial stewardship, and routine microbiological surveillance. Rapid and accurate detection methods are essential for early identification and containment of potential outbreaks [18]. In the present study, polymerase chain reaction (PCR) analysis targeting the *bla_{OXA-51}* gene confirmed the identity of the majority of the isolates as *Acinetobacter baumannii*. The high detection rate of this intrinsic gene highlights the reliability of PCR as a sensitive and specific method for accurate identification of this pathogen. The consistent amplification of the target gene among clinical isolates indicates its widespread presence in the studied setting and underscores its diagnostic significance. The application of PCR in this study proved to be a rapid and dependable approach compared to conventional identification methods, which may occasionally yield ambiguous results. Accurate molecular confirmation not only facilitates appropriate clinical management but also supports effective infection control by ensuring correct identification of the pathogen involved. Such molecular approaches play a crucial role in strengthening diagnostic precision and guiding preventive strategies in high-risk environments such as burn units [19][20].

Conclusion

The present study highlights the usefulness of molecular techniques, particularly polymerase chain reaction (PCR), for the accurate identification of *Acinetobacter baumannii*. PCR provides a rapid and highly specific method for confirming the presence of this organism, thereby improving the reliability of laboratory diagnosis. The isolates obtained from burn patients underline the clinical significance of this pathogen in a highly vulnerable group, where factors such as loss of skin integrity, prolonged hospital stay, and frequent invasive interventions increase the risk of infection. These findings emphasize the need for continuous microbiological monitoring and the strict implementation of infection prevention and control practices to limit the spread of multidrug-resistant strains. Strengthening diagnostic accuracy through molecular methods can contribute to better patient management and help reduce morbidity, mortality, and healthcare-associated transmission in burn care settings.

Limitation of work

This study has certain limitations. Being conducted in a single tertiary care burn unit with a cross-sectional design, the findings may have limited generalizability and may not adequately capture temporal variations or the full extent of genetic diversity. Although polymerase chain reaction (PCR) was employed for molecular identification, the use of more advanced typing approaches such as pulsed-field gel electrophoresis (PFGE), multilocus sequence typing

(MLST), or whole-genome sequencing could offer higher discriminatory resolution. Furthermore, the molecular analysis in this study was restricted to selected genetic targets and did not include a comprehensive assessment of antimicrobial resistance determinants, virulence-associated factors, or potential environmental reservoirs. Future multicenter, longitudinal studies incorporating advanced genomic techniques are therefore recommended to achieve a more detailed understanding of *Acinetobacter baumannii* epidemiology in burn care settings.

Risk Factor

Burn patients are highly vulnerable to *Acinetobacter baumannii* infection due to loss of the protective skin barrier, extensive or full-thickness burns, and large TBSA involvement. Prolonged hospitalization, ICU stay, invasive procedures, and repeated surgeries further increase the risk of nosocomial acquisition. Prior exposure to broad-spectrum antibiotics promotes multidrug-resistant strains, while burn-induced immunosuppression enhances susceptibility. Additionally, environmental contamination and inadequate infection control practices facilitate cross-transmission within burn units.

Profit of the Outcome

The findings of this study have important clinical and public health implications. Molecular identification of *Acinetobacter baumannii* using polymerase chain reaction (PCR) allows for rapid and precise diagnosis, facilitating timely initiation of appropriate antimicrobial therapy. This improved diagnostic accuracy supports better clinical decision-making and enhances patient management. Additionally, these results contribute to strengthening infection prevention and control measures, as well as antimicrobial stewardship practices within the burn unit. Effective implementation of these strategies can help reduce morbidity, mortality, duration of hospitalization, and overall healthcare costs, while improving patient safety in high-risk burn care settings.

Acknowledgement

The authors are thankful to the technical staff in the hospital laboratories for helping in performing the investigations.

Author' contributions

SR was responsible for data analysis, manuscript drafting, review and editing, and funding acquisition. RK and KS were responsible for study conception and design.

Funding

Research is self-funded by SUM Hospital, Bhubaneswar, Odisha, India Siksha 'O' Anusandhan University, Bhubaneswar, Odisha, India.

Competing Interests

The authors declare that they have no competing interests.

Ethical Compliance

There was no direct involvement of any human or animalmodel in this study. Institute Ethical Committee approval number is Ref.no/ICE/IMS.SH/SOA/2024/892.

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