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Research Article

Molecular detection of blaOXA-23, csuE and ompA genes from carbapenem-resistant and biofilm producing *Acinetobacter baumannii* isolated from clinical samples

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Abstract

Acinetobacter baumannii has become a critical hospital pathogen due to its biofilm formation and multidrug resistance, particularly against carbapenems. This study aimed to detect blaOXA-23, ompA and csuE genes in carbapenem-resistant, biofilm-producing *A. baumannii* in clinical isolates. Among 521 clinical samples, 39 MDR and carbapenem-resistant isolates were selected. Antibiotic susceptibility was tested via Kirby-Bauer disc diffusion with carbapenemase production confirmed by the combined-modified carbapenem inactivation method. Biofilm production was assessed by congo red agar and modified microtiter plate assay. PCR was used to detect blaOXA-23, ompA and csuE genes. All isolates were resistant to ceftazidime and ampicillin but sensitive to colistin. Of the 39 isolates, 29 produced biofilms (10 strong, 7 moderate, 12 weak and 10 non-biofilm producers). The prevalence of blaOXA-23, ompA and csuE genes was 61.53%, 41.02%, and 46.15%, respectively with a significant correlation between antibiotic resistance, biofilm formation and gene presence. The high prevalence of MDR *A. baumannii* with biofilm-associated genes and carbapenem resistance in hospitals highlights the need for stringent control measures and regular monitoring.

Keywords: *Acinetobacter baumannii*, Biofilm formation, blaOXA-23, csuE, MDR, ompA

Introduction

Antibiotic resistance has become a global issue, severely limiting the treatment of common infectious diseases (Nemec et al., 2016). Once considered a low-level pathogen, *Acinetobacter baumannii* has emerged as a significant cause of nosocomial infections, particularly septicemia and pneumonia in immunocompromised ICU patients. Its persistence in hospital environments and ability

to form biofilms make it a growing concern worldwide (Almasaudi, 2018, Gedefie et al., 2021).

Acinetobacter baumannii is known for its biofilm-forming ability and resistance to multiple antibiotics, which complicates treatment (Zeighami et al., 2019; Roy et al., 2022). Carbapenem-resistant *A. baumannii* (CRAB) is associated with various carbapenemase classes and international clones, making infection management challenging (Doi et al., 2009; Peleg et al., 2007). Carbapenem resistance mechanisms include carbapenemase production, reduced membrane permeability, altered penicillin-binding proteins, and efflux pump overexpression (Joshi et al., 2017). Among these, blaOXA-23 is the most prevalent gene, especially in Asian countries (Poirel et al., 2010; Peleg et al., 2008; Turton et al., 2006).

The World Health Organization (WHO) has labelled *A. baumannii* a "Priority Organism" due to its rapid spread of antibiotic resistance through biofilm formation (Gedefie et al., 2021). Biofilm production involves complex mechanisms such as collagen addition, pili expression, iron acquisition and quorum sensing (Gaddy & Actis, 2009; Tomaras et al., 2003). Virulence factors linked to biofilm formation include ompA, csuE, Bap, PNAG, and others (Ghasemi et al., 2018; Thummeepak et al., 2016).

The ompA gene contributes to cell adhesion, biofilm formation, and immune response modulation (Asif et al., 2018; Cassin & Tseng, 2019). The csuE gene, part of the csu operon, is essential for pilus formation and biofilm development (Cincarova et al., 2016; Tomaras et al., 2008). Studies in Taiwan and Nepal have reported high prevalence rates of csuE and ompA genes, with significant correlations between antibiotic resistance, biofilm formation and associated genes (Yang et al., 2019; Shrestha et al., 2015).

In Nepal, *A. baumannii* isolates have shown high carbapenem resistance rates, with blaOXA-23 being the most common gene detected. Studies have also reported biofilm-producing *A. baumannii* from various clinical settings, including 53.97% at B.P. Koirala Institute of Health Sciences and 14% at Shree Birendra Hospital (Baniya et al., 2019; Dumaru et al., 2019; Yadav et al., 2017). This study aimed to investigate the antimicrobial susceptibility, carbapenemase production, and biofilm-related genotypes of carbapenem-resistant *A. baumannii* isolated from clinical samples in Nepal.

Materials and Methods

Sample collection and processing

This descriptive cross-sectional study was conducted from August 2022 to February 2023 at the Annapurna Neurological Institute and Allied Sciences, Kathmandu, in collaboration with Golden Gate International College, Kathmandu. Samples were collected from both inpatients and outpatients of all age groups with suspected bacterial infections. Ethical approval was obtained from the Institutional Review Committee (Reg no: 011/2022) at Annapurna Neurological Institute and Allied Sciences. Written informed consent was obtained from all participants.

A total of 521 clinical samples (blood, urine, sputum, catheter tips, CSF, tracheal aspirates and CVP tips) were analyzed. *A. baumannii* isolates were identified through standard microbiological techniques, including gram staining, biochemical tests (catalase, oxidase, citrate, urease and TSI), and cultural characteristics (Cheesbrough, 2006).

Antibiotic susceptibility testing (AST)

AST was performed by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar according to CLSI guidelines (2022). Antibiotic discs used included ampicillin, amikacin, gentamycin, ceftazidime, cotrimoxazole, levofloxacin, meropenem, imipenem, piperacillin-tazobactam and colistin.

Biofilm and carbapenemase detection

Biofilm production was assessed using the congo red agar (CRA) method and modified microtiter plate assay (Stepanovic et al., 2007). Carbapenemase production was evaluated using the combined-modified carbapenem inactivation method (mCIM and eCIM) following CLSI protocols (Vander et al., 2015).

Table 1: Specific primers used in the study for the amplification of target genes.

Target gene	Primer	Sequence	Size / annealing temperature
*bla*OXA-23	*bla*OXA-23^{F}	5' GATCGGATTGGAGAACCAGA 3'	501 bp / 52℃
	*bla*OXA-23^{R}	5' ATTTCTGACCGCATTTCCAT 3'	
OmpA	*ompA*$^{-F}$	5' CTGGTGTTGGTGCTTTCTGG 3'	352 bp / 49℃
	ompA^{-R}	5' GTGTGACCTTCGATACGTGC 3'	
CsuE	*csuE*$^{-F}$	5' ATGCATGTTCTCTGGACTGATGTTGAC 3'	976 bp / 60℃
	csuE^{-R}	5' CGACTTGTACCGTGACCGTATCTTGATAAG 3'	

Molecular analysis

DNA extraction was performed by the phenol:chloroform method. The PCR reaction mixture (15 µl) was prepared as follows; for the blank, 7.5 µl PCR water, 6.5 µl master mix and 0.5 µl each of forward and reverse primers, for the positive control, 4.5 µl PCR water, 3 µl positive control, 6.5 µl master mix and 0.5 µl each of forward and reverse primers, and for the sample, 4.5 µl PCR water, 3 µl genomic DNA, 6.5 µl master mix and 0.5 µl each of forward and reverse primers were added. PCR amplification was performed using genomic DNA under thermal cycling conditions as described by Li et al. (2014) for blaOXA-23, Ghasemi et al. (2018) for ompA and Seifi et al. (2016) for csuE (Table 1). The amplified products were characterized by 2% agarose gel electrophoresis in tris-acetate-EDTA with 0.6 µl ethidium bromide as a tracking dye. After solidification, 2 µl of 100 bp DNA ladder, 3 µl positive control, 3 µl negative control and 3 µl PCR products were loaded into the wells (Figure 1-3).

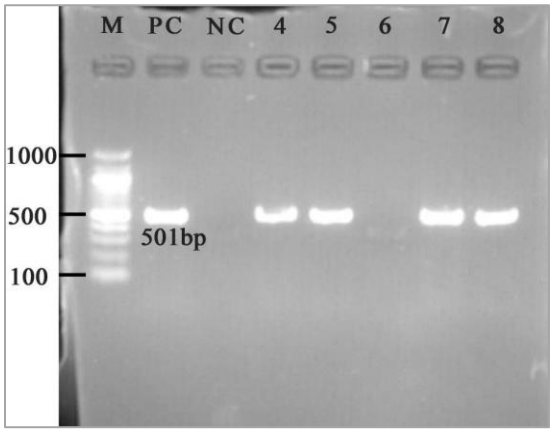

Figure 1: Gel Electrophoresis; Lane M: 100 bp ladder, lane PC: positive control, lane NC; negative control, lane 4, 5, 7 and 8 positive samples for blaOXa-23 gene and lane 6 negative samples with band appearing at 501 bp.

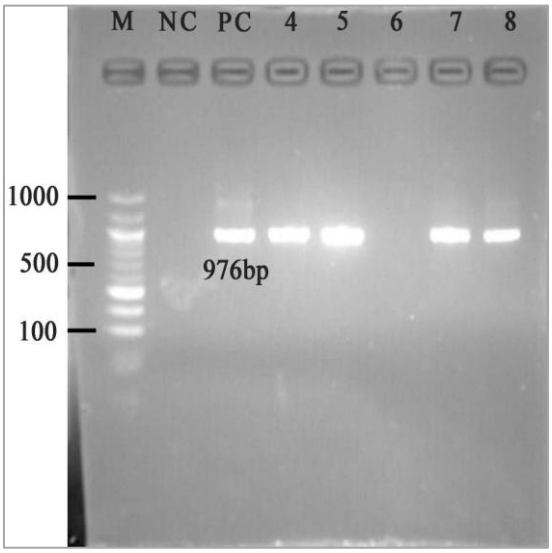

Figure 2: Gel Electrophoresis; Lane M: 100 bp ladder, lane NC: negative control, lane PC; positive control, lane 4, 5, 7 and 8 positive samples for csuE gene and lane 6 negative samples with band appearing at 976 bp.

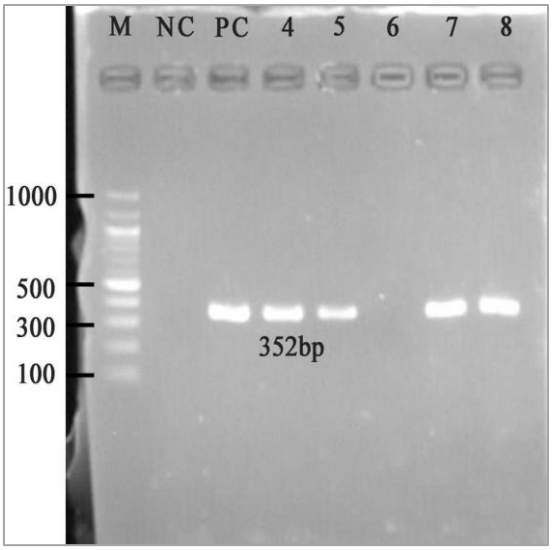

Figure 3: Gel Electrophoresis; Lane 1: 100 bp ladder, lane 2: negative control, lane 3; positive control, lane 4, 5, 7 and 8 positive samples for ompA gene and lane 6 negative samples with band appearing at 352 bp.

Statistical analysis

Data were analyzed using R software. Chi-square and regression analysis were applied to determine associations between variables with significance set at $p \leq 0.05$.

Results and Discussion

Among 521 clinical samples, 154 (29.55%) showed growth, and *A. baumannii* accounting for 25% (39/154) of isolates. Out of 39 isolates of *A. baumannii*, the majority of *A. baumannii* was isolated from sputum specimen 48.71% (19/39) followed by CSF 15.38% (6/39), urine 10.25% (4/39), pus 7.69% (3/39), CVP tips, tracheal aspirates, wound swab 5.12% (2/39), and throat swab 2.56% (1/39) while no *A. baumannii* isolates were isolated from blood, synovial fluid, tissue and pleural fluid specimens. No isolates were detected from blood, synovial fluid, tissue or pleural fluid.

Infections were more prevalent in male patients (58.97%) than female patients (41.02%). The highest prevalence was in patients aged 16–32 years (25.64%). The majority of *A. baumannii* isolates were from ICU patients (51.28%), followed by the post-operative ward (25.64%) and general ward (23.07%).

All isolates were resistant to ampicillin and ceftazidime (100%), with high resistance also observed against imipenem (97.43%), levofloxacin (94.87%), meropenem (92.30%) and gentamycin (92.30%). All isolates were sensitive to colistin. Phenotypic tests showed 36/39 (92.30%) isolates as carbapenemase producers by AST and 25/39 (64.10%) by mCIM. A significant association was observed between mCIM and blaOXA-23 gene occurrence ($p < 0.05$). Using the modified microtiter plate assay, 29 isolates (74.36%) were biofilm producers: 10 strong, 7 moderate and 12 weak. The congo red agar method identified 25 strong biofilm producers (64.10%).

Among 25 carbapenemase producers, 7 were strong, 5 were moderate, 8 were weak, and 5 were non-biofilm producers. A significant correlation was found between biofilm formation and carbapenem resistance ($p = 0.0069$) (Table 2). Prevalence of genes: blaOXA-23 (61.53%), ompA (41.02%) and csuE (46.15%) have shown significant associations between the occurrence of these genes, carbapenem resistance and biofilm formation ($p < 0.05$) (Table 3).

Table 2: Association between carbapenem resistance and biofilm production.

Biofilm production	**Carbapenemase producer**	**Carbapenemase non-producer**	**Total**	**p-value**
	Number (%)	Number (%)	Number (%)	
Strong	7 (17.94)	3 (7.69)	10 (25.64)	
Moderate	5 (12.82)	2 (5.12)	7 (17.94)	
Weak	8 (20.51)	4 (10.25)	12 (30.76)	0.0069
Non-producer	5 (12.82)	5 (12.82)	10 (25.64)	
Total	**25 (64.09)**	**14 (35.88)**	**39 (100)**	

Table 3: Comparison of combined-modified carbapenem inactivation method with bla_{OXA-23}.

Combined-modified carbapenem inactivation method	***blaOXA*-23**		**Total**	**p-value**
	Positive	Negative		
Positive	24	1	25	
Negative	0	14	14	0.0253
Total	**24**	**15**	**39**	

Table 4: Association between biofilm formation and biofilm related genes.

Biofilm Formation	**Isolates / Biofilm formation (%)**	**Biofilm-related genes isolates / Genes (%)**	
		ompA	*csuE*
Non-biofilm	10 (25.64)	0.00	2 (5.12)
Weak biofilm	12 (30.76)	5 (12.82)	5 (12.82)
Moderate biofilm	7 (17.94)	3 (7.69)	2 (5.12)
Strong biofilm	10 (25.64)	8 (20.51)	9 (23.07)
Total	**39 (100)**	**16 (41.02)**	**18 (46.15)**
p-value		0.004	0.008

The presence of both ompA and csuE genes is more common in strong biofilm producers, suggesting a potential link between these genes and the ability to form stronger biofilms. The statistically significant p-values indicate a significant association between biofilm formation and the presence of both genes ($p < 0.05$) (Table 4).

This study highlights the high prevalence of multidrug-resistant (MDR) *A. baumannii* and its association with biofilm formation and carbapenem resistance. The presence of blaOXA-23, ompA and csuE genes among isolates indicates a strong correlation between genetic factors, antimicrobial resistance and biofilm production.

The dominance of *A. baumannii* in respiratory samples (48.71%) aligns with previous studies associating it with ventilator-associated pneumonia (Yadav et al., 2020; Shrestha et al., 2015). The high prevalence in ICU patients (51.28%) further supports its role as a major nosocomial pathogen. Antibiotic susceptibility testing revealed complete resistance to ampicillin and ceftazidime, with high resistance to carbapenems (imipenem and meropenem), consistent with previous reports from Nepal and other countries (Joshi et al., 2017; Ghimire et al., 2021). The 100% susceptibility to colistin highlights its effectiveness as a last-resort treatment for *A. baumannii* infections.

Phenotypic tests identified 92.30% of isolates as carbapenemase producers, and 64.10% were confirmed via mCIM. This discrepancy indicates that mCIM may not detect all carbapenemase-producing strains. The blaOXA-23 gene was present in 61.53% of isolates, consistent with its role as a predominant carbapenem resistance gene in Asian countries (Poirel et al., 2010). Biofilm formation was detected in 74.36% of isolates by the modified microtiter plate assay. Strong biofilm producers were mostly associated with carbapenem resistance, confirming the protective role of biofilms against antibiotics (Ghasemi et al., 2018). The congo red agar method was effective in detecting strong biofilm producers but less sensitive in distinguishing moderate and weak biofilm producers.

The ompA and csuE genes were detected in 41.02% and 46.15% of isolates, respectively. These genes are essential for biofilm formation and play a significant role in enhancing antibiotic resistance. The substantial correlation between biofilm formation, carbapenem resistance, and these genes suggests that targeting biofilm-related genes may be an effective therapeutic strategy. The findings support the urgent need for improved infection control measures and regular surveillance of MDR *A. baumannii*. Effective monitoring of carbapenem resistance genes and biofilm-related genes could help in designing better strategies to combat this pathogen.

Conclusion

This study demonstrates a high prevalence of MDR *A. baumannii* isolates associated with carbapenem resistance and biofilm formation. The majority of the isolates harboured the blaOXA-23 gene, confirming its role as a key determinant of carbapenem resistance in Nepal. Significant associations were found between carbapenem resistance, biofilm formation, and the presence of ompA and csuE genes. The findings emphasize the need for enhanced infection control measures, periodic surveillance of resistance genes, and strategic use of effective antibiotics like colistin to

manage infections. Targeting biofilm-related genes could be a promising approach to combatting *A. baumannii* infections.

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