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Biofilm formation capacity and Carbapenem-resistance in *Acinetobacter-calcoaceticus-baumannii* isolated from inpatients in a tertiary care hospital in Nepal

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Abstract

Objective *Acinetobacter calcoaceticus-baumannii* complex (ACBC), as an emerging global burden to various clinical infections, has a huge problem in empirical therapy due to the increasing resistance to the majority of antibiotics. The ability of biofilm formation added to its antimicrobial resistance and helped its persistence and survival in the environment. To associate biofilm formation with carbapenem resistance, a hospital-based cross-sectional study was carried out from February 2020 to August 2020 at Kathmandu Model Hospital, Kathmandu, Nepal. ACBC was identified from the clinical samples following standard Microbiological procedures. A modified Kirby-Bauer disk diffusion method was performed to assay the antibiotic susceptibility testing of ACBC isolates to various antibiotic classes. A quantitative adherence assay was used to determine the biofilm assay. A conventional Polymerase Chain Reaction (PCR) method was used to find the targeted biofilm-related genes, *Bap*, *csuE*, and bla_{PER1} using specific primers.

Results Out of 665 different clinical samples, bacterial growth was observed in 281 (42.3%) clinical samples. Of these, 32 (11.4%) isolates were identified as ACBC. Out of 32 ACBC isolates, 29 (90.6%) of which were carbapenem-resistant. All carbapenem-resistant ACBC isolates were found to be sensitive to Polymixin B and Colistin. Out of 29 CR-ACBC, 17.2% of isolates were resistant to Tigecycline. The majority of ACBC isolates (93.8%) were multidrug-resistant (MDR) while 13 (40.6%) of isolates were extensively drug-resistant (XDR). A total of 31 ACBC isolates were biofilm producers, out of which 2 were strong biofilm producers followed by 8 moderate, and 21 were weak biofilm producers. The occurrence of biofilm-forming genes; *Bap*, *csuE*, and bla_{PER1} genes were found to be 65.6%, 65.6%, and 56.3% respectively among ACBC clinical isolates. A significant association was observed between carbapenem resistance, biofilm formation, and biofilm-related genes.

Conclusion The higher rate of MDR and XDR ACBC isolates associated with biofilm formation in the study alarms the ACBC-related infection in clinical settings among inpatients. The hospital environment and clinical equipment

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are potential sources of biofilm-forming isolates. Hence, the effective sterilization of clinical equipment and hospital environment are utmost and a strong policy should be made to prescribe the proper antibiotic based on antibiogram profile to fight against an emerging threat of ACBC infections.

Keywords *Acinetobacter baumannii*, Biofilm, Carbapenem-resistant, Biofilm related genes; *Bap*, *CsuE*, And bla_{PER1}

Introduction

Acinetobacter calcoaceticus-baumannii complex (ACBC) is a Gram-negative commensal bacterium that often infects immunocompromised patients or patients with indwelling devices, especially in the intensive care unit (ICU) [1]. It causes a wide range of hospital-acquired infections such as respiratory tract infections, urinary tract infections, bacteremia, sepsis, endocarditis, meningitis, skin and soft tissue infections, burns as well as central and nervous system infections [2].

A. baumannii infections have become a public health threat due to the rapid rise in the incidence of multidrug-resistant (MDR) strains [3]. Carbapenem are choice of drug to treat severe infections caused by MDR *A. baumannii* due to its good activity and low toxicity [4]. However, the prevalence of carbapenem-resistant *A. baumannii* has increased globally limiting the treatment options and leading to increased mortality of patients [5]. The pathogen developed resistance to carbapenems due to the acquisition of antibiotic-resistant genes, decreased permeability, altered penicillin-binding protein, overexpression of efflux pump, production of carbapenemase enzyme mainly class B metallo beta-lactamases (MBLs) and class D oxacillinase and biofilm formation [5–6]. World Health Organization (WHO) has listed carbapenem-resistant *A. baumannii* as a top priority pathogen requiring additional study and development of new antibiotics in 2017 [7].

A. baumannii is an emerging pathogen with the ability to produce a biofilm that mostly causes device-related infections such as ventilator-associated pneumonia and catheter-related infections [8]. The bacteria inside the biofilm are shielded by EPS which acts as a barrier so antibiotics cannot penetrate it leading to antibiotic-resistant [9]. Biofilm-forming bacteria show 1000-fold higher drug resistance than planktonic cells. So, biofilm-related infections are chronic, prone to relapse, and more difficult to treat [10]. Within biofilm communities, the bacterial cells are in close proximity and have a high chance of horizontal gene transfer (HGT), particularly via conjugation of antibiotic resistance genes, promoting their survival and spread of antibiotic resistance [11].

Biofilm-related virulence factors involved in *A. bauamnnii* infections are biofilm-associated protein (*Bap*), extended-spectrum beta-lactamase (ESBL) family bla_{PER1} gene, and CsuA/BABCDE pilus usher-chaperone assembly system [12]. The *bap* gene encodes a biofilm-forming protein that is crucial for bacterial cell accumulation, intracellular adhesion, and biofilm formation in both biotic and abiotic surfaces [13]. *CsuE* is a component of the *csuA/BABCDE* chaperon-usher assembly system that is required to form a pilus-like bundle during the initial attachment phase of biofilm formation and mainly aids in biofilm formation in abiotic surfaces [14]. The presence and expression of the bla_{PER1} promote biofilm formation as well as respiratory epithelial cell adhesion in clinical isolates of *A. baumannii* [15]. To address the biofilm-associated carbapenem-resistant *A. baumannii* infections among hospitalized patients, we conducted a hospital-based cross-sectional study at a Tertiary Care Hospital in Kathmandu, Nepal. We primarily determined the rate of *A. baumannii* in different clinical specimens. We have also evaluated the association between biofilm formation with carbapenem-resistant ACBC isolates detecting biofilm-forming genes *Bap*, *csuE*, and bla_{PER1}.

Methods

Study design, study site, and study population

A hospital-based cross-sectional study was conducted at Kathmandu Model Hospital, Kathmandu, Nepal from February 2020 to August 2020. The study population were the inpatients admitted to the hospital during the study period.

Inclusion and exclusion criteria

All age groups of both sexes admitted to Kathmandu Model Hospital, Kathmandu who gave written consent were enrolled in this study.

Sample type and sample size

The clinical samples include pus, sputum, tracheal aspirates, blood, endotracheal tips, catheter tips, wound samples, suction tips, and tissue. A total of 665 clinical samples were collected and further processed in this study.

Bacterial identification and antibiotic susceptibility testing (AST)

The samples were inoculated on Mac Conkey Agar (MA) and Blood Agar (BA) (Hi-media) and incubated at 37 °C for 24 h and 48 h respectively. The ACBC isolates were identified by standard Microbiological procedures including Gram staining, culture, and various biochemical tests. *Acinetobacter baumannii* was phenotypically identified as Gram-negative coccobacilli that gives positive results with catalase, methyl red, citrate and

10% lactose utilization tests. However, it gives negative results with oxidase, indole, Voges-Proskauer, hemolysis, nitrate reduction, urease, gas and H_2S production tests. It is non-fastidious, non-fermentative (oxidative) and can grow at 44 °C. A total of 32 clinical isolates of ACBC were obtained. The antibiotic susceptibility pattern of ACBC was determined by a modified Kirby-Bauer disk diffusion method following CLSI guideline 2020 [16]. The antibiotics used in the study were; Amoxicillin (30 µg), Cefotaxime (30 µg), Levofloxacin (5 µg), Nitrofurantoin (300 µg) (for urine samples only), Cotrimoxazole (25 µg), Amikacin (30 µg), Gentamycin (10 µg), Ofloxacin (5 µg), Ciprofloxacin (5 µg), Amoxicillin/calvulant (30 µg), Ceftazidime (30 µg), Doxycycline (30 µg), Piperacillin (100 µg), Piperacillin tazobactam (100/10µg), Chloramphenicol (30 µg), Meropenem (10 µg), Imipenem (10 µg), Ertapenem, Cefepime (30 µg), Cefoperazone sulbactam (75/30µg), Ampicillin/sulbactam (10/10µg), Polymyxin B(300U), Colistin (30 µg) and Tigecycline (15 µg) (Himedia). The isolates that were resistant to at least 1 agent in ≥ 3 antimicrobial categories were considered MDR [17]. Likewise, the isolate that was non-susceptible to ≥ 1 agent in all but ≤ 2 categories was considered extensively drug-resistant (XDR) [17]. Based on the criteria, the prevalence of MDR and XDR were also determined.

Detection of biofilm by microtitre plate method

The biofilm formation on the microtitre plate was performed according to the protocol described by Stepanovic et al., 2007. The optical density (OD) at 630 nm was measured using a micro-titer reader(Bio-tek). The assay was carried out at least three times to obtain average optical density [18]. The optical density cutoff value (ODc) was calculated by using the given formula and the results were interpreted as ODc = average OD of negative control + (3 × SD of the negative control), Non-biofilm producer: OD ≤ ODc; Weak biofilm producer: ODc < OD ≤ 2×ODc; Medium biofilm producer: 2×ODc < OD ≤ 4×ODc and Strong biofilm producer: 4× ODc ≤ OD.

Table 1 Distribution of bacterial isolates among positive cultures

Bacterial isolates	Number (%)
E. coli	81 (28.8)
S. aureus	57 (20.3)
K. pneumoniae	46 (16.4)
ACBC	32 (11.4)
P. aeruginosa	23 (8.1)
E. faecalis	11 (3.9)
E. faecium	2 (0.7)
C. freundii	11 (3.9)
S. pneumoniae	1 (0.4)
P. mirabilis	11 (3.9)
P. vulgaris	4 (1.4)
S. saprophyticus	1 (0.4)
E. aerogenes	1 (0.4)
Total	281 (100)

Detection of *Bap*, *CsuE* and bla_{PER1}

The genomic DNA was extracted by using a genomic extraction kit (Qiagen). The primer used for biofilm genes detection were *bap*-F(5'-TGCTGACAGTGACG TAGAACCACA-3'), *bap*-R(5'-TGCAACTAGTGGAAT AGCAGCCCA-3'), *csuE*-F(5'-CATCTTCTATTTCGG TCCC-3'), *csuE*-R (5'-CGGTCTGAGCATTGGTAA-3'), and bla_{PER1}-F (5'- GCAACTGCTGCAATACTCGG-3'), bla_{PER1}-R (5'-ATGTGCGACCACAGTACCAG-3'). The PCR was performed using Qiagen (USA) Master mix in ProFlex thermal cycler. The conditions for PCR amplification were initial denaturation at 95 °C for 15 min, followed by 35 cycles of denaturation at 94 °C for 30 s, primer annealing at 57 °C for *bap*, 59 °C for *csuE* for 30 s and extension at 72 °C for 30 s whereas 40 cycles of denaturation at 94 °C for 60 s, primer annealing at 50 °C for 1 min, an extension at 72 °C for 45 s for bla_{PER1} followed by a final extension at 72 °C for 5 min. PCR end products were analyzed by electrophoresis in 1.5% agarose gel. The expected amplified product size for *bap*, *csuE*, and bla_{PER1} genes were 184 bp, 168 bp, and 900 bp respectively [13].

Quality control

A. baumannii ATCC 17,978 was used as a positive control for the biofilm-producing isolate.

Data analysis

SPSS version 21 was used for data entry and statistical analysis. The correlation between biofilm formation and carbapenem resistance was analyzed by using the Chi-Square Test. *P*-value < 0.05 was considered significant.

Ethical approval

Ethical approval for the study was taken by the International Review Committee (IRC) Phect Nepal (003-2020).

Results

Distribution of bacterial isolates among positive cultures

A total of 665 clinical specimens from inpatients were received and processed in the microbiological lab of KMH during the study period. Among 665 samples, growth was observed in 281 (42.3%) clinical samples while 384 (57.7%) showed no growth. Out of 281 isolates, *E. coli* (28.8%) was the most predominant pathogen followed by *S. aureus* (20.3%), *K. pneumoniae* (16.4%), ACBC (11.4%), *P. aeruginosa* (8.1) and others (Table 1).

Gender-wise and age-wise distribution of *Acinetobacter calcoaceticus baumannii* complex (ACBC)

A significantly higher incidence of ACBC infection was observed among the male patients (26/32; 81.3%) as compared to female patients (6/32; 18.7%). Similarly, the highest incidence of ACBC infection was reported in the patient of age group 20–50 years which was more than half percent (59.6%) followed by the patient of age group 60–70 (15.5%), 50–60 (12.5%), 10–20 (6.2%) and 70–80 years (6.2%).

Distribution of *Acinetobacter calcoaceticus baumannii* complex (ACBC) in various clinical specimens

Of 665 clinical samples processed, 32 isolates were identified as ACBC. The highest number of ACBC was isolated from pus samples ($n = 12$, 37.5%) followed by tracheal aspirates ($n = 6$, 18.8%), and sputum ($n = 3$, 9.4%) samples (Fig. 1).

Antibiotic susceptibility pattern of ACBC isolates

All ACBC isolates were resistant to Amoxicillin, Cefotaxime, and Ceftazidime whereas 31 isolates were resistant to Amikacin and Gentamycin antibiotics. Most of the isolates were susceptible to Doxycycline (53.1%) followed by Cotrimoxazole (18.7%), Levofloxacin (15.6%), and Ofloxacin (15.6%). The respiratory ACBC was highly resistant to Azithromycin, a common antibiotic used in respiratory tract infections. All isolates were susceptible to Colistin and Polymyxin B (Table 2).

Rate of MDR and XDR ACBC isolates

The majority of ACBC isolates 30 (93.8%) were MDR while 13 (40.6%) isolates were XDR 5 of the XDR were only susceptible to the Polymixin category and 8 were sensitive to Polymixin and Tigecycline categories.

Association between carbapenem resistance and biofilm production

Out of 32 isolates, 2 isolates were strong biofilm producers, 8 isolates were moderate, and 21 were weak biofilm producers. Only one isolate was the non-biofilm producer. A significant association was observed between carbapenem resistance and biofilm formation (p-value < 0.05) indicating the role of biofilm in carbapenem resistance (Table 3).

Distribution of biofilm-associated genes

Out of 32 clinical isolates of ACBC, 31 were biofilm positive, and biofilm-related virulence genes *Bap* and *csuE* were detected in 67.8% of clinical isolates whereas the frequency of bla_{PER1} was found to be 58%. No genes were detected in the non-biofilm-producing isolate (Table 4; Figs. 2 and 3).

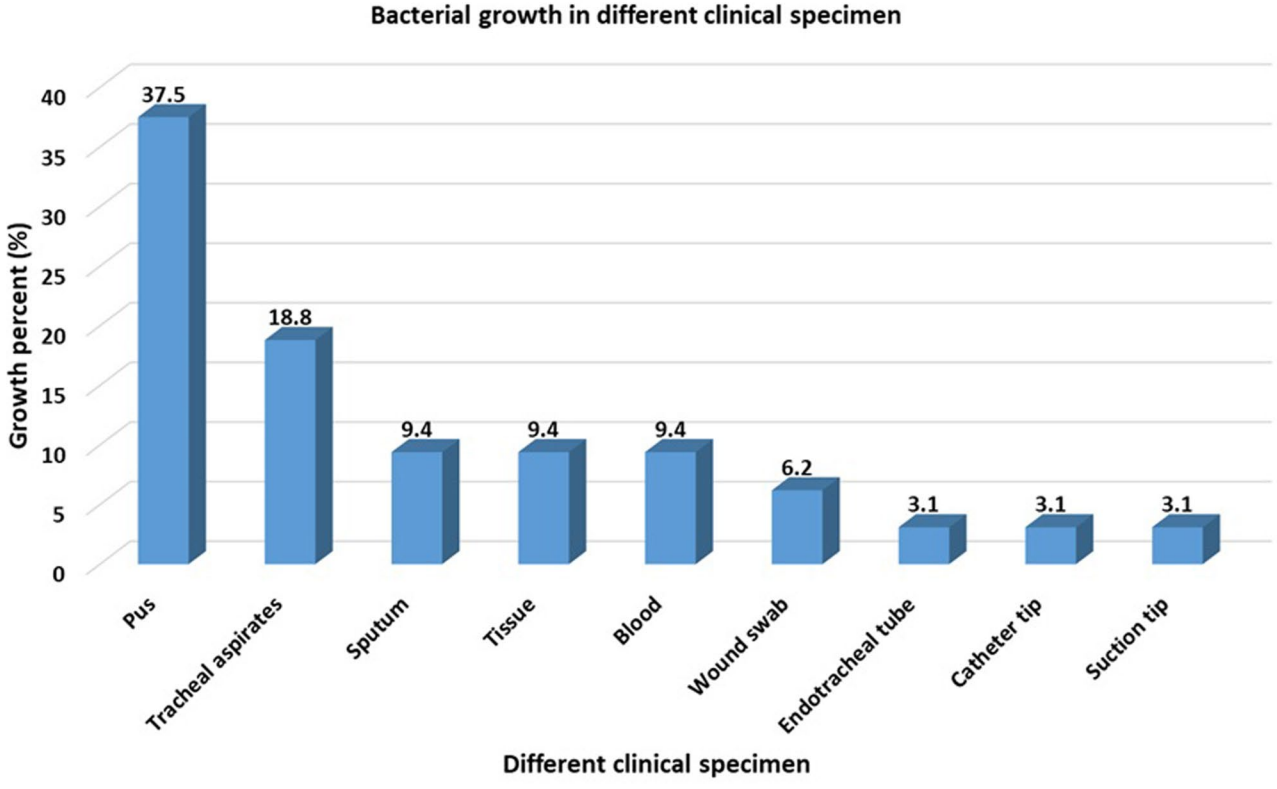

Fig. 1 Distribution of ACBC among different clinical specimens

Table 2 Antibiotic susceptibility testing of ACBC isolates ($n=32$)

Antibiotic category	Antibiotics used	No. of ACBC isolates (%)	
		Susceptible *N* (%)	Resistant *N* (%)
First Line Drugs			
Penicillin + β-lactamase inhibitors	Amoxicillin	0	32 (100)
	Ampicillin/sulbactam	3 (9.4)	29 (90.6)
Extended-spectrum cephalosporins; 3rd and 4th generation cephalosporins	Cefixime[a]	2 (15.4)	11 (84.6)
	Cefotaxime	0	32 (100)
	Ceftazidime	0	32 (100)
Folate pathway inhibitors	Cotrimoxazole	6 (18.7)	26 (81.3)
Macrolides	Azithromycin[b]	1 (10)	9 (90)
Aminoglycosides	Gentamycin	1 (3.1)	31 (96.9)
Fluoroquinolones	Ciprofloxacin	4 (12.5)	28 (87.85)
	Levofloxacin	5 (15.6)	27 (84.4)
	Ofloxacin	5 (15.6)	27 (84.4)
Phenicols	Chloramphenicol	2 (6.2)	30 (93.8)
Second Line Drugs			
Aminoglycosides	Amikacin	1 (3.1)	31 (96.9)
Penicillin + β-lactamase inhibitors	Amoxicillin-clavulanic acid	2 (6.2)	30 (93.8)
Extended-spectrum cephalosporins; 3rd and 4th generation cephalosporins	Cefperazone/Sulbactam	2 (6.2)	30 (93.8)
	Cefepime	2 (6.2)	30 (93.8)
Tetracyclines	Doxycycline	17 (53.1)	15 (46.9)
Carbapenems	Imipenem	3 (9.4)	29 (90.6)
	Meropenem	3 (9.4)	29 (90.6)
	Etrapenem	3 (9.4)	29 (90.6)
β-lactamase inhibitors	Piperacillin/Tazobactam	2 (6.2)	30 (93.8)
Third/last Line Drugs			
Polymyxins	Colistin	32 (100)	0
	Polymyxin B	32 (100)	0
Glycylcyclines	Tigecycline	27 (94.4)	5 (15.6)

Note: The susceptibility assay for Cefixime[a] was used for only blood ($n=3$) and respiratory ACBC isolates ($n=10$). Likewise, the susceptibility testing for Azithromycin[b] was used for respiratory ACBC isolates ($n=10$)

Table 3 Association between carbapenem resistance and biofilm production

Biofilm Production	Number of Carbapenem		Total	*p*-value
	Resistant (%)	Sensitive (%)		
Strong	2 (6.2)	0	2 (6.2)	0.013
Moderate	8 (25)	0	8 (25)	
weak	19 (59.4)	2 (6.2)	21 (65.6)	
Non	0	1 (3.1)	1 (3.1)	
Total	29	3	32 (100)	

Note: The optical density cutoff value (ODc) was calculated by using the given formula and the results were interpreted as ODc = average OD of negative control + (3 × SD of the negative control), Non-biofilm producer: OD ≤ ODc; Weak biofilm producer: ODc < OD ≤ 2×ODc; Moderate biofilm producer: 2×ODc < OD ≤ 4×ODc and Strong biofilm producer: 4× ODc ≤ OD. *Chi-square test

Table 4 Distribution of biofilm-associated genes *Bap*, *CsuE* and bla_{PER1}

Biofilm-producing gene →	Number of isolates with biofilm-producing genes (%)		
	Bap	csuE	bla_{PER1}
Positive	21 (65.6)	21 (65.6)	18 (56.2)
Negative	11 (34.4)	11 (34.4)	14 (43.8)
Total	32 (100)	32 (100)	32 (100)

Association between carbapenem resistance and biofilm-related genes (*Bap*, *CsuE* and bla_{PER1})

Significant associations between carbapenem-resistant and biofilm-related genes *Bap*, *csuE*, and bla_{PER1} were observed (p-value < 0.05) which indicates the role of biofilm-related genes in antibiotic resistance to carbapenem (Table 5).

Co-existence of *Bap*, *csuE*, and bla_{PER1} genes

Out of 31 biofilm-positive isolates, 21 isolates were positive for both *Bap* and *csuE* genes and 18 isolates were positive for the bla_{PER1} gene. The higher co-existence of all three genes i.e. *Bap*, *csuE*, and bla_{PER1} (58%) was observed among the isolates. (Fig. 4).

Correlation between biofilm-related genes with biofilm formation

All three genes *Bap*, *csuE*, and bla_{PER1} were present in strong and moderate biofilm-producing isolates. A significant correlation between biofilm-forming genes *Bap*, *csuE*, and bla_{PER1} and biofilm intensity was found (Table 6).

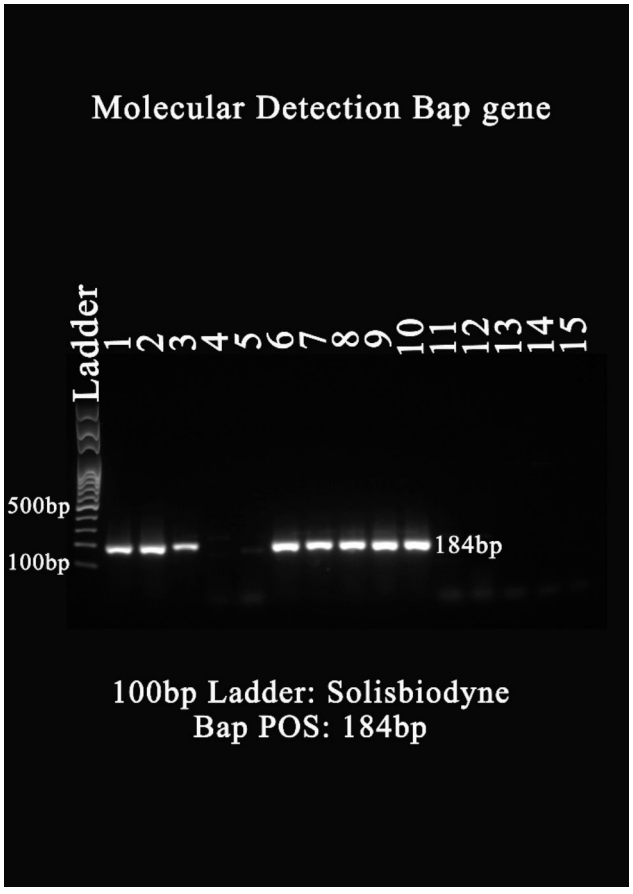

Fig. 2 Detection of *Bap* genes in ACBC isolates by conventional PCR (Ladder, L1: positive control, L2– L13: amplified products from ACBC isolates, L14: negative control, L15: no template control)

Fig. 3 Detection of *csuE* genes in ACBC isolates by conventional PCR (Ladder, L1: positive control, L2– L13: amplified products from ACBC isolates, L14: negative control, L15: no template control)

Table 5 Association between carbapenem resistance and biofilm-related genes

Carbapenem	Biofilm related gene		Total	*p*-value
	Detected (%)	Not detected (%)		
Sensitive	0	3 (100)	**3**	0.033
Resistance	21 (72.4)	8 (27.6)	**29**	
Total	**21**	**11**	**32**	

Discussion

Acinetobacter baumannii infections have become a public health threat due to bacteria's growing resistance to all available antibiotics [19]. Because of its ability to produce biofilm, it is very challenging to treat and control the infection in hospital settings [11]. In our study, out of 665 in-patient clinical specimens, 281 showed positive bacterial growth and 32 (11.4%) were ACBC which accounts for the third most commonly isolated Gram-negative bacilli following *E. coli* and *K. pneumoniae.* Similar results were also published by Khanal et al. and Raut et al. [20–21]. In our previous study at Manipal Teaching Hospital, Pokhara, we observed ACBC infections ($n=117$) as the primary cause of nosocomial infections followed by *P. aeruginosa* infections ($n=79$) [22]. The majority of isolates were identified from pus and respiratory samples including tracheal aspirates, sputum, and ET tube. In the study conducted in TUTH, the majority of ACBC was detected from respiratory samples (47.2%), followed by pus and swabs (27.3%) and body fluids (11.1%) [23].

In our previous studies, we had reported an increasing antimicrobial resistance among Gram-negative bacteria from various infections including bacterial isolates from immunocompromised patients [24–26]. Among Gram-negative bacteria, *Acinetobacter* shows higher resistance to commonly used antibiotics. In this study, 100% ACBC isolates were resistant to Amoxicillin and third-generation cephalosporins (Ceftazidime, Cefotaxime, and Ceftriaxone), 97% ACBC isolates were resistant to Gentamycin and Amikacin and followed by fluoroquinolones, Chloramphenicol, Cotrimoxazole and β-lactamase inhibitors. Yadav et al. and Gurung et al. presented similar reports from the study conducted in Nepal [23, 27]. Carbapenem is used to treat MDR ACBC infections but a high prevalence of CR-ACBC (91%) was observed in this study which was consistent with a previous study

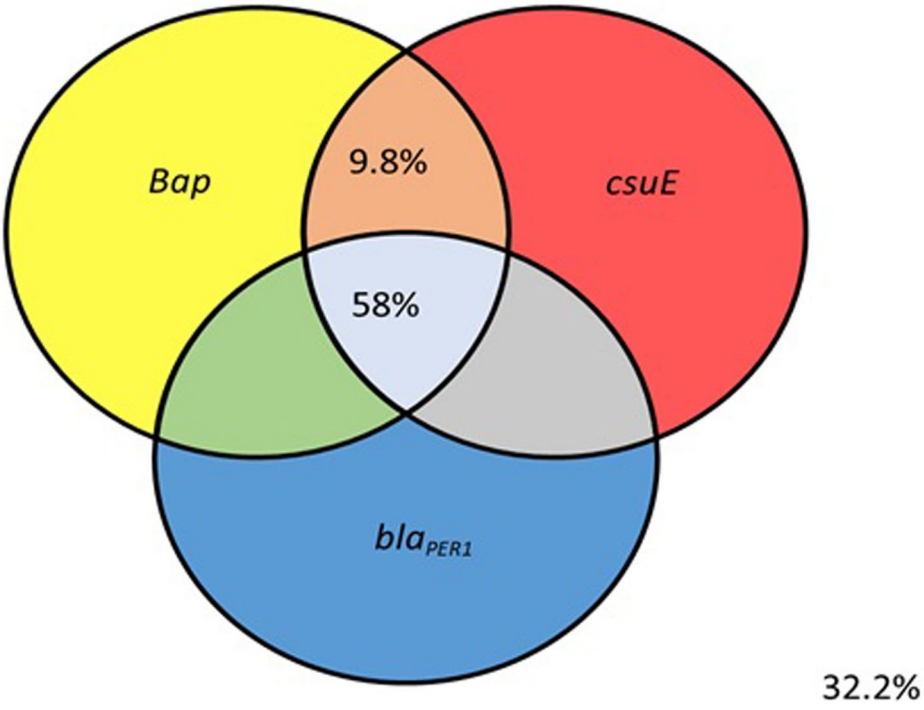

Fig. 4 Co-existence of *Bap*, *csuE* and bla_{PER1} genes

Table 6 Association between biofilm forming genes with biofilm intensity

Biofilm Intensity (Number of biofilm-producing isolates)	ACBC isolates possessing biofilm-related genes (%)			*p*-value
	Bap	*csuE*	bla_{PER1}	
Strong ($n=2$)	2 (100)	2 (100)	2 (100)	0.032
Moderate ($n=8$)	8 (100)	8 (100)	8 (100)	
Weak ($n=21$)	11 (52.4)	11 (52.4)	8 (38.1)	
Non ($n=1$)	0	0	0	
Total ($n=32$)	**21 (65.6)**	**21 (65.6)**	**18 (56.2)**	

done by Joshi et al. [28]. In contrast to our result, Mahto and Dhungel reported 61.2% and 50.5% imipenem and meropenem resistance respectively [29]. In another study in a tertiary care hospital at Kathmandu, Nepal, 87.9% ACBC isolates were found to be carbapenem-resistant [30]. This indicates the rise of CR ACBC infections in Nepal in recent years. The higher rate of antimicrobial resistance in bacterial pathogens is due to the irrational use of antibiotics, adherence to empirical therapy without proper AST, extensive use of antibiotics in poultry, and, direct disposal of antimicrobial waste in the environment [31, 32]. High antibiotic susceptibility of ACBC isolates towards doxycycline antibiotics was reported so it might be used to treat MDR ACBC infections. Carbapenem resistance in *A. baumannii* is mainly caused by class B MBL and class D OXA type β-lactamase which can hydrolyze carbapenem antibiotics [33]. CR-AB infections have a high morbidity and death rate in hospital settings due to their low level of antibiotic susceptibility and subsequent failure of therapy [34]. In this study, all ACBC isolates were susceptible to Colistin and Polymyxin B whereas 15.6% of isolates were resistant to Tigecycline by disk diffusion method. Yadav et al. also reported 100% susceptibility of ACBC to Colistin and Polymyxin B which supported the findings of this study [35]. Hence, these can be the choice of drugs for the effective treatment of CR-ACBC-associated infections.

A. baumannii has a high biofilm-forming ability which poses serious public health problems because of biofilm-associated resistance to available antimicrobial agents leading to a huge challenge in hospital settings [8]. In this study, 31 (96.9%) were biofilm producers and only one was non biofilm producer. Among these 2 were strong, 8 were moderate and 21 were weak biofilm producers. Similar rates of biofilm-forming *Acinetobacter* species were also reported by Khanal et al. (99%) and Bhandari et al. (91.6%) from tertiary care hospitals in Nepal [20, 36]. In this study, biofilm-producing ACBC isolates were more resistant to carbapenem than non-biofilm producers indicating the role of biofilm in antimicrobial resistance. This study shows a significant correlation between biofilm formation and carbapenem resistance which was in accordance with previous studies by Anish et al., Pattanaik and Banashankari, and Sunu Kumari et al. [6, 37, 38]. The increase in antimicrobial resistance among biofilm producer *A. baumannii* is due to the slow growth rate inside biofilm as well as its mechanical and biochemical

shield such as low O_2, high CO_2, high pH, low water, and nutrient availability leading to poor penetration of antibiotics [39]. Inside the biofilm, the bacteria are near each other and there is a high chance of horizontal gene transfer via the conjugation of resistant genes which promotes their survival in the presence of antibiotics [9, 40].

The prevalence of *Bap*, *csuE*, and bla_{PER-1} genes was found to be 65.6%, 65.6%, and 56.3% respectively. In another study, the prevalence of *Bap*, *csuE*, and bla_{PER-1} were 79.2%, 38.3%, and 91.6% respectively, and significant correlation between antibiotic resistance, biofilm formation, and related genes [13] which was in support of our study. Similar types of findings were reported by Yadav et al. from a study conducted at a tertiary care hospital in Nepal i.e. 90.0% and 46.6% for *csuE*, *and bap* respectively [35]. The presence of biofilm-related genes in all types of clinical specimens in our study indicates biofilm-related genes help in biofilm formation, survival in hospital environments and medical devices as well as disease pathogenesis in hospital settings. No biofilm-related genes were found in carbapenem-sensitive ACBC isolates and a significant association between carbapenem resistance and biofilm-forming genes *bap*, *csuE*, and bla_{PER-1} was found. Further, the co-existence of *Bap*, *csuE*, and bla_{PER1} among positive biofilm isolates was found to be 58% which may have boosted biofilm formation. The co-existence of *Bap* and *csuE* was 9.8% and no genes were singly present which also indicates the dependence of genes on biofilm formation such as *csuE* is critical for initial attachment and *bap* for biofilm maturation.

Conclusion

The higher rate of multidrug resistant and extensively drug-resistant ACBC from different clinical specimens demonstrated complete resistance to the majority of antibiotics, however, Colistin, Polymyxin 'B' and Tigecycline were still found to be effective drugs to treat CR ACBC infections. The increase in biofilm formation significantly associated with carbapenem resistance adds a big challenge to controlling CR ACBC infections. Hence, proper sterilization (using effective antimicrobial agents on hospital floors, contaminated surfaces and possibly indoor air) of hospital environment and clinical equipment should be of primary concern and a strong policy to prescribe effective antibiotics based on antibiogram profile should be implemented.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13104-025-07211-5.

Supplementary Material 1

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Author contributions
SB, MKU, and UTS developed the protocol, collected and analyzed data, and wrote the manuscript. KBA and BS supervised laboratory work at the hospital. UTS supervised Molecular work. UTS and BS were the main reviewers of the manuscript. All authors reviewed and approved the final version of the manuscript to submit for publication.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study was approved by the International Review Committee (IRC) Phect Nepal (003-2020). Written informed consent was obtained from all the participants and/or their legal guardians. All experiments were performed in accordance with relevant guidelines and regulations (such as the Declaration of Helsinki).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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