Progress in Microbes and Molecular Biology

HH PUBLISHER

Original Research Article

Biofilm Formation and *bla*$_{OXA}$ Genes Detection Among *Acinetobacter baumannii* from Clinical Isolates in a Tertiary Care Kirtipur Hospital, Nepal

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Article History

Received: 30 September 2021;

Received in Revised Form: 30 October 2021;

Accepted: 1 November 2021;

Available Online: 9 November 2021

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Abstract: (1) Background*: Acinetobacter baumannii* has emerged as a leading cause of nosocomial infections as they are capable of evolving resistance to various classes of antibiotics. The ability of *A. baumannii* to form biofilm might also be associated with increased antibiotic resistance and hence treatment failure. This study was carried to associate the biofilm formation with the drug resistance pattern of *A. baumannii* and to detect *bla*$_{OXA-23}$, *bla*$_{OXA-24}$, and *bla*$_{OXA-51}$ from carbapenem resistance isolates. (2) Methods: Among different clinical samples, a total of 19 *Acinetobacter* spp. were identified with conventional microbiological procedures. The biofilm production was determined by a quantitative adherence assay. The antimicrobial susceptibility test was carried out by the Kirby-Bauer disc diffusion method, carbapenemase production detection was confirmed by Modified Hodge Test, and target resistant genes were detected by polymerase chain reaction. (3) Results: Out of 90 clinical specimens, 64.44% (58/90) showed bacterial growth, whereas 32.75% (19/58) isolates were identified as *A. baumannii*. Among all *A. baumannii* isolates, 84.21% (16/19) were multidrug-resistance and 63.16% (12/19) carbapenem resistance phenotypically. *bla*$_{OXA-51}$ was detected in all the isolates and *bla*$_{OXA-23}$ was detected only in 63.16% (12/19) isolates. However, *bla*$_{OXA-24}$ was not detected in any of the isolates. Among *A. baumannii*, 89.47% (17/19) isolates produced biofilm with 47.37% (9/19) strong biofilm producers. (4) Conclusions: In the majority of MDR *A. baumannii*, *bla*$_{OXA-51}$ and *bla*$_{OXA-23}$ were detected as the determinant factor for carbapenem resistance having a direct relation with biofilm formation. This study provided a valuable clue for the management of *A. baumannii* infections in clinical settings.

Keywords: *A. baumannii*; MDR; biofilm; carbapenem resistance; blaOXA genes

1. Introduction

Acinetobacter, a ubiquitous, non-fermentative, Gram-negative bacteria, persisting in the hospital environment, is a nosocomial pathogen responsible for a wide range of infections[1,2]. It is an opportunistic pathogen causing wound infections, peritonitis, endocarditis, cholangitis, ventilator-associated pneumonia (VAP), bacteremia, ICU infections, and urinary tract infections (UTIs), particularly in patients with a severe health condition[3]. The emergence of *Acinetobacter* as a significant nosocomial and opportunistic pathogen is influenced by its survival ability, ubiquitous presence, and instant progress of resistance to the commonly used antimicrobials[4,5]. The high level of *A. baumannii* resistance to antibiotics is a result of its capacity to produce carbapenemase and to form biofilms[6]. Carbapenemase-producing bacteria receive particular consideration as it is linked with multidrug-resistance (MDR) having a limited antibiotic choice. However, resistance to these agents is now considered as World Health Organization's number one critical priority pathogen[7]. The underlying antibiotic resistance mechanisms may be related to efflux pump overexpression, decreased permeability, and carbapenemase production. Among the carbapenemases, carbapenem-hydrolyzing class D β-lactamases (CHDLs) are considered the most prevalent cause of carbapenem resistance in *A. baumannii*[8]. The CHDLs in *A. baumannii* can be grouped into six subclasses: intrinsic chromosomal OXA-51-like, acquired OXA-23-like, OXA-24/40-like, OXA-58-like, OXA-143-like, and OXA235-like β-lactamases[9]. The intrinsic $bla_{\text{OXA-51}}$ gene is characteristic of this species and is usually weakly expressed[10]. Though, it can illustrate that carbapenem resistance increases its expression when an insertion sequence IS*Aba1* precedes the gene by providing promoter sequences[11]. The $bla_{\text{OXA-23}}$ gene was first categorized in Scotland which has been progressively reported worldwide. *Acinetobacter radioresistens* was recently recognized as the progenitor of the $bla_{\text{OXA-23}}$-like genes. Sequence comparisons from different groups of OXA-type enzymes, OXA-23, OXA-24, and OXA-58, which can be plasmid or chromosomally encoded, have been most often linked to carbapenem-resistant clinical strains of *A. baumannii*, but OXA-23 has the highest distribution worldwide[8]. Various studies conducted in Nepal have confirmed and reported different groups of OXA-type enzymes in *A. baumannii*. A university hospital, Kathmandu, reported 80.3% carbapenem-resistant MDR *A. baumannii* where all isolates harbored $bla_{\text{OXA-51-like}}$, most of them had $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-420}}$ (a novel $bla_{\text{OXA-58}}$ variant), and 36% isolates had $bla_{\text{OXA-104}}$[12]. Similarly, in another study conducted at a tertiary hospital, Nepal, 98% carbapenem-resistant MDR *A. baumannii* were reported. This study showed that all the isolates contained $bla_{\text{OXA-23}}$ whereas $bla_{\text{OXA-24}}$

and *bla*$_{\text{OXA-58}}$ were not detected in any of the analyzed isolates[13]. Besides, previous studies have reported varying prevalence of carbapenem resistance in bacteria in different settings, including 9.09% in Shahid Gangalal National Heart Centre, Kathmandu[14], 25.7% in Nepal Medical College, Kathmandu[15], 56% in Shree Birendra Hospital, Kathmandu[16], and 28% in Paropakar Maternity and Women's Hospital, Kathmandu[17]. The prevalence of carbapenem-resistant strains varies in different hospitals, laboratories, and research centers among the samples used for the isolation of bacteria. *A. baumannii* has been identified as a "red-alert" human pathogen due to the rapid progress of multiple antibiotic resistance contributed by biofilm formation as well[18]. Biofilm is a structured community of microorganisms enclosed in a self-produced polymeric matrix adherent to an inert or living surface[19,20]. Biofilm formation is phenotypically related to exopolysaccharide (EPS) production, nutrient availability, bacterial surface components (outer membrane proteins, adhesins), quorum sensing, and pilus formation[21,22]. Formation of biofilm helps *Acinetobacter* easily survive and transfer in hospital environments such as attachment to various biotic and abiotic surfaces, e.g., vascular catheters, cerebrospinal fluid shunts, or Foley catheters[23]. Biofilm is a relevant process for bacterial survival due to its mechanism for antibiotic resistance, transfer of resistance plasmids, and a medium for intercellular communication[24]. Among *Acinetobacter* spp., biofilm-producing strains are immensely resistant to antimicrobial agents than those that do not produce biofilm[24]. The mechanism responsible for antimicrobial resistance in biofilm-producing organisms may be delayed penetration of antimicrobial agents through the biofilm matrix, trapping of the antibiotics in the exopolysaccharide matrix, an altered growth rate of biofilm organisms, and other physiological changes due to the biofilm mode of growth[25]. Biofilm formation by these bacteria has been their protective weapon for continuing their survival in a wide range of environmental conditions. This increases a prominently therapeutic question for the treatment of nosocomial infections throughout the globe.

In Nepal, previous studies have reported varying prevalence of biofilm-producing *A. baumannii* in different settings, including 14% in Shree Birendra Hospital, Kathmandu[16], 53.97% in BPKIHS, Dharan[26], and 51.11% in Annapurna Neurological Institute and Allied Science, Kathmandu[27]. The rising incidence of antibiotic resistance in *A. baumannii* is a matter of great concern to mankind. This study is crucial for understanding the occurrence and distribution of the biofilm-producing multidrug-resistant *A. baumannii* in the Nepalese clinical environment and the association between carbapenem resistance and the presence of *bla*$_{\text{OXA}}$ genes in *A. baumannii.*

2. Materials and Methods

2.1. Study Design, Study Site, and Sample Population

This was a hospital-based cross-sectional study conducted from March to September 2019. The clinical specimens were collected and processed at Phect-Nepal Kirtipur Hospital, Kathmandu, and DNA extraction and polymerase chain reaction were carried out at the Central Department of Microbiology, Tribhuvan University. The study population included patients of all age groups and both genders.

2.2. Sample Collection and Transportation

All the clinical specimens, including wound swab, pus, blood, urine, sputum, catheter tips, tissue, and body fluids, were collected by experienced medical personnel in a clean, leak-proof, sterile container according to the specimen type as per the guidelines of the hospital. The collected specimens were immediately transferred without any delay to the microbiology laboratory for routine culture and antibiotic sensitivity testing[28,29].

2.3. Culture and Identification of Bacterial Isolates

Each clinical specimen was inoculated onto blood agar and MacConkey's agar plates and incubated aerobically for 24 hours at 35˚C. Individual colonies were identified by standards microbiological tests as per the morphology, gram staining, and various biochemical tests which were catalase test, oxidase test, Voges Proskauer test, Methyl Red test (MR-VP), citrate test, urease test, oxidative-fermentative test, TSI (Triple Sugar Iron agar) test[28,29,30].

2.4. Antibiotic Susceptibility Test of A. baumannii

Bacterial isolates were analyzed for antibiotic susceptibility tests using the modified Kirby-Bauer disc diffusion method as stated by Clinical Laboratory Standards Institute guidelines (28). The antibiotics used in the study were amoxicillin/clavulanic acid (30/10 µg), amikacin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefepime (30 µg), cotrimoxazole (25 µg), ciprofloxacin (5 µg), gentamicin (10 µg), imipenem (10 µg), meropenem (10 µg), piperacillin-tazobactam (100 µg), tetracycline (30 µg), polymyxin B (300 µg), colistin (10 µg), doxycycline (30 µg). Multidrug resistance was defined as resistance to three or more classes of the antimicrobials tested[31].

2.5. Screening for Carbapenemase Production

For screening of carbapenemase production, bacterial isolates resistant to imipenem and meropenem were selected. If the inhibition zone was ≤19 mm for imipenem and ≤19 mm for meropenem, the isolates were subjected to tests for confirmation of carbapenemase[31].

2.6. Phenotypic Confirmation of Carbapenemase Producers

Modified Hodge Test (MHT) was used for phenotypic confirmation of the carbapenemase producers[31]. For the test, *E. coli* ATCC 25922 in 5 ml Mueller-Hinton broth (MHB) was prepared corresponding to 0.5 McFarland standard. It was then diluted 1:10 by adding 0.5 ml of broth culture to 4.5 ml of MHB. A lawn culture of diluent was prepared on MHA and allowed to dry for 3-5 minutes. A 10 µg meropenem disc was placed on the center of the test area. In a straight line, *A. baumannii* was streaked from the edge of the disc to the edge of the plate at 3 different places. The plate was incubated overnight at 35℃ in ambient air for 16-24 hours. They were examined for a clover leaf-type indentation or flattening at the intersection of the *A. baumannii* and the *E. coli* 25922 within the zone of inhibition of the carbapenem susceptibility disc.

2.7. Detection of bla$_{OXA}$

The genomic DNA was extracted from *A. baumannii* by using the protocol as described by Pu *et al*[32]. For the detection of *bla*$_{\text{OXA-23}}$, *bla*$_{\text{OXA-24,}}$ and *bla*$_{\text{OXA-51}}$ the following primers were used; OXA-23F (5'-GAT CGG ATT GGA GAA CCA GA-3'), OXA-23R (5'-ATT TCT GAC CGC ATT TCC AT-3') with expected PCR product of 501 bp; OXA 24F (5'-GGT TAG TTG GCC CCC TTA AA-3'), OXA-24R (5'-AGT TGA GCG AAAAGG GGA TT-3') with expected PCR product of 246 bp; OXA-51F (5'-TAA TGC TTT GAT CGG CCT TG-3'), OXA-51R (5'-TGG ATT GCA CTT CAT CTT GG-3') with expected PCR product of 353 bp[33]. The conditions used were as follows: 5 min at 94°C, followed by 30 cycles of 45 s at 94°C, 1 min at 52°C, and 1 min at 72°C, and a final extension of 5 min at 72°C. The amplified products were visualized on 1% agarose gel, containing ethidium bromide.

2.8. Biofilm Formation Assay

The biofilm-forming ability of the bacterial isolates was determined by using a quantitative adherence assay[34]. Each isolate was cultured overnight in trypticase soy broth (TSB) at 37℃. 2 µl of cell suspension was inoculated in sterile 96 well polystyrene microtiter plates with 198 µl of TSB. After 24 h of incubation at 37℃, the wells were gently washed three times with 200 µl of phosphate-buffered saline (PBS), then dried in an inverted position and stained with 50 µl of 0.1% crystal violet. Subsequently, the wells were gently washed three times with 200 µl of distilled water and dried in an inverted position. The wells were added with 200 µl of 5% isopropanol to solubilize the residual crystal violet. The optical density (OD) at 570 nm was determined using a microtiter plate reader. Each isolate was tested by using several wells (repeated 8-12 wells), and the average optical density was

obtained. Optical density cut-off (ODc) was defined as an average OD of negative control + (3 × standard deviations (SD) of negative control).

The interpretation of biofilm production was done according to the following criteria[35].

• Non-biofilm producer: OD ≤ ODc

• Weak biofilm producer: 2×ODc < OD ≤4×ODc

• Medium biofilm producer: 2×ODc < OD ≤4×ODc

• Strong biofilm producer: OD > 4×ODc

2.9. Data Analysis

All the data obtained were analyzed using the statistical programming Statistical Package of Social Sciences (SPSS version 21.0). A Chi-square test was used to determine the association of independent variables and a p-value ≤ 0.05 was considered statistically significant.

2.10. Ethics Statement

Ethical approval for this study was obtained from the Institutional Review Committee (IRC), Phect-Nepal (004-2019). Written informed consent was obtained from each patient for their voluntary participation in the study.

3. Results

3.1. Distribution of A. baumannii Isolates

A. baumannii was detected in 19 of 90 non-duplicate clinical isolates (21.11%). The majority of *A. baumannii* was identified in wound swabs (*n*=6, 31.58%), tissue (*n*=4, 21.05%), sputum (*n*=3, 15.79%), and pus (*n*=2, 10.52%), followed by catheter tips (*n*=1, 5.26%), blood (*n*=1, 5.26%), urine (*n*=1, 5.26%), and body fluid (*n*=1, 5.26%). Among 19 isolates of *A. baumannii*, 84.21% were MDR of which 26.31% isolated from wound swabs followed by tissue (21.05%). Similarly, 89.47% of the samples produced carbapenemase, with the greatest levels detected in wound swab (*n*=6, 31.58%), tissue (*n*=3, 15.78%), sputum (*n*=3, 15.78%), and pus (*n*=2, 15.78%) (Figure 1).

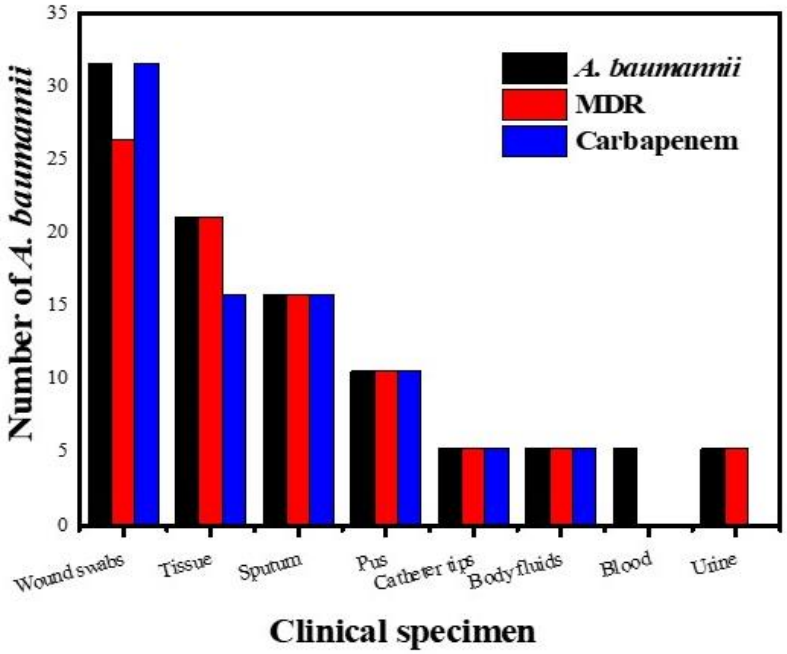

Figure 1. Distribution of *A. baumannii*, MDR, and Carbapenem among different clinical specimen

3.2. Antibiotic Susceptibility Pattern of A. baumannii

All isolates were susceptible to colistin and tetracycline but resistant to cefotaxime and ceftazidime. Most of them were resistant to Amoxicillin/clavulanic acid (94.74%), Cefepime (73.68%), Imipenem (89.48%), Meropenem (89.48%), Cotrimoxazole (89.48%), Ciprofloxacin (83.6%), Amikacin (84.21%), Gentamicin (89.48%), and Doxycycline (89.48%) (Figure 2).

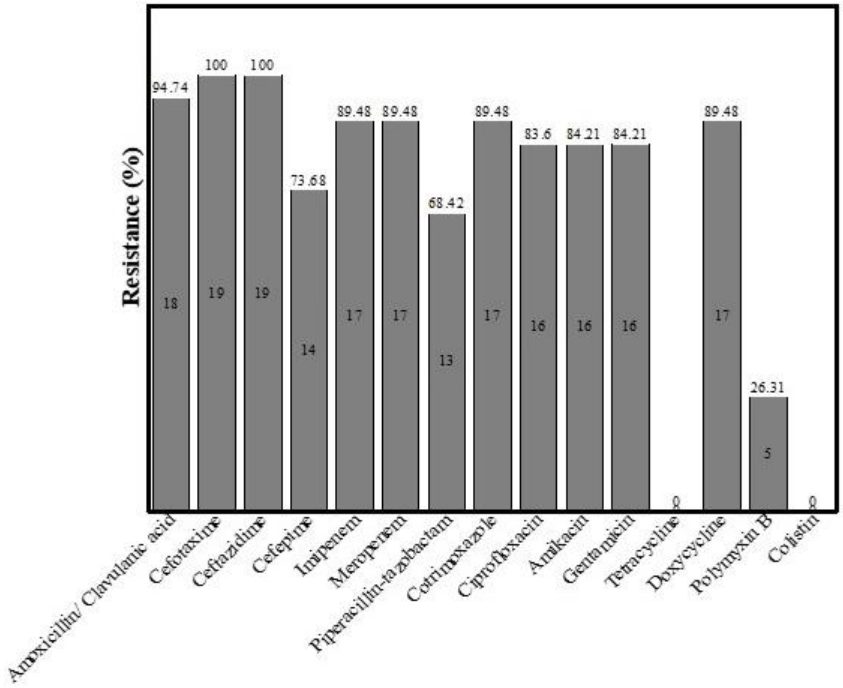

Figure 2. Antibiotic susceptibility pattern of *A. baumannii*

3.3. Association Between Biofilm Formation and MDR Acinetobacter Isolates

The microtiter plate technique was used to assess biofilm formation. Nine strains (47.37%) of *A. baumannii* isolates are strong biofilm producers, five strains (26.31%) are moderate biofilm producers, three strains (15.79%) are weak biofilm producers, and two strains (10.53%) are non-biofilm producers. MDR was detected in 84.21% of *A. baumannii* isolates. A statistically significant relationship between MDR *A. baumannii* and biofilm generation capability was discovered ($p \leq 0.005$) (Table 1).

Table 1. Association between biofilm formation and MDR *Acinetobacter* isolates

Biofilm production	**MDR** **N (%)**	**Non-MDR** **N (%)**	**Total** **N (%)**	***p*-value***
Strong	9 (47.37)	0	9 (47.37)	
Moderate	4 (21.05)	1 (5.26)	5 (26.31)	
Weak	3 (15.79)	0	3 (15.79)	0.005
Non-producer	0	2 (10.53)	2 (10.53)	
Total	16 (84.21)	3 (15.79)	19 (100)	

*Chi-square test

3.4. Association Between Biofilm Formation and Carbapenemase-producing Acinetobacter Isolates

Among 12 carbapenemase producers, (n=8, 42.10%) were strong biofilm producers and (n=3, 21.05%) were moderate biofilm producers. A significant statistical association (p<0.043) had been observed between biofilm formation ability and carbapenemase production (Table 2).

Table 2. Association between biofilm formation and phenotypic carbapenemase-producing *Acinetobacter* isolates

Biofilm production	**Carbapenemase producers** **N (%)**	**Carbapenemase non-producers** **N (%)**	**Total** **N (%)**	***p*-value***
Strong	8 (42.10)	1 (5.26)	9 (47.37)	
Moderate	3 (21.05)	2 (10.53)	5 (26.31)	
Weak	1 (5.26)	2(10.53)	3 (15.79)	0.0043
Non-producer	0	2 (10.53)	2 (10.53)	
Total	12(63.15)	7 (36.83)	19 (100)	

*Chi-square test

3.5. Prevalence of bla$_{OXA}$ Genes in A. baumannii Isolates

Of the 19 *A. baumannii*, all isolates harbored *bla*$_{\text{OXA-51}}$, none of the isolates carried *bla*$_{\text{OXA-24}}$ and 63.2% isolates had *bla*$_{\text{OXA-23}}$. The *bla*$_{\text{OXA-51}}$ and *bla*$_{\text{OXA-23}}$ co-occurred in 63.2% of *A. baumannii* isolates. However, all 3 genes did not co-occur in a single isolate of *A. baumannii* (Figure 3).

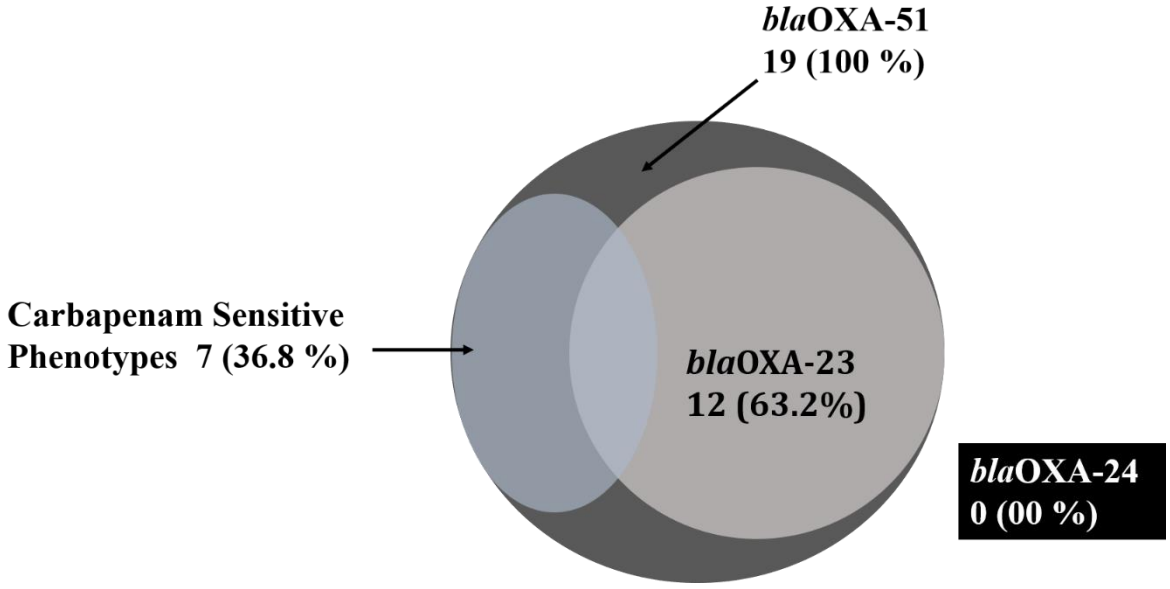

Figure 3. Venn diagram showing concurrence of OXA genes in *A. baumannii* isolates

4. Discussion

Acinetobacter species are ubiquitous organisms and prevail in natural environments. Transmission of an isolate is usually through the hands of staff, contaminated equipment, or the overall hospital environment. The prevalence of *A. baumannii* was 21.11% (19/90) in this study which is similar to some previous studies reported from Nepal[36,37]. As the pathogen can endure dry conditions for a long time, they persist in the hospital environment and are responsible for opportunistic infections[17]. Hence, the highest number of isolates were isolated from in-patients (84.2%) which is following other studies[38,49].

All isolates of *A. baumannii* were resistant to cefotaxime and ceftazidime, nearly 90% of the isolates were resistant to imipenem and meropenem, and more than 80% were resistant to cotrimoxazole, amikacin, and gentamicin. However, all the isolates were susceptible to colistin and tetracycline. These are the deliberated narrow alternatives for the treatment of carbapenem-resistance *A. baumannii*. Also, nearly 85% of *A. baumannii* isolates were found to be multidrug-resistance. Other studies conducted in Nepal have reported 97.9%[13] and 82.8%[15] *A. baumannii* to be MDR. The increased antibiotic resistance of *A. baumannii* is most probably due to the extensive misuse of antimicrobial agents in Nepal[40]. The

prevalence of carbapenem resistance in *Acinetobacter* in UK, USA, Singapore, Chile, Korea, Portugal, India, and Pakistan was 47-77%, 48%, 50%, 70%, 92%, 85%, 40% and 62-100%, respectively[41-46]. The development of resistance in *A. baumannii* may be due to the presence of wide array β -lactamases that hydrolyze and confer resistance to penicillins, cephalosporins, and carbapenems[17]. Resistance to carbapenem is facilitated by resistance mechanisms including enzymatic inactivation, active efflux of drugs, and modification of target sites[47]. Among meropenem-resistance *A. baumannii* isolates, 63.16% were carbapenemase producers and all phenotypic carbapenemase producers carried at least one or more carbapenemase genes as shown by PCR. Similarly, the study carried out in Morocco in 2019 showed that 69.6% were positive for carbapenemase production by MHT[48]. Following CLSI 2018, MHT is no longer recommended as a phenotypic test for carbapenemase detection presumably because of the poor sensitivity of the test when detecting certain extended-spectrum β-lactamase synthesis associated with porin loss[49]. In the current investigation, however, all isolates identified as carbapenemase producers by MHT also had one or more carbapenemase genes, as shown by PCR. Results of the current study (*bla*$_{\text{OXA-51}}$ (100%), *bla*$_{\text{OXA-23}}$ (63.15%)), are consistent with those of other studies, which had reported the most prevalent carbapenem hydrolyzing β-lactamases genes in *A. baumannii* to include *bla*$_{\text{OXA-51}}$ (83–100%) and *bla*$_{\text{OXA-23}}$ (59–96%)[50-52]. Therefore, the most prevalent mechanism underlying the resistance of *A. baumannii* to carbapenem antibiotics is the production of OXA-type β-lactamases which can hydrolyze carbapenem antibiotics and also be considered as the main factor that causes multidrug resistance in *A. baumannii*.

Furthermore, *bla*$_{\text{OXA-24}}$ was not detected in any isolates in this study. The *bla*$_{\textit{OXA-24}}$ gene is common in *A. baumannii* isolated from European countries[53]. This result is similar to the study conducted in Brazil[54] and the United States of America[55]. The previous studies from Nepal did not detect this gene too[12,13]. However, the *bla*$_{\text{OXA-24}}$ gene was reported in some studies in Taiwan[56], Iran[57], Poland[58], and France[59]. Altogether, these results suggest a specific distribution of *bla*$_{\text{OXA}}$ gene variants in different regions.

To the best knowledge, this study revealed the highest co-occurrence of *bla*$_{\text{OXA-51/23}}$ in *A. baumannii* isolates from Nepal and demonstrated the increase of co-occurrence of *bla*$_{\text{OXA-51/23}}$ overtime in Nepal. The results of this study are consistent with the previous studies that report high carbapenem resistance in *A. baumannii* strains from Nepal with OXA-type carbapenemase predominancy[60,61]. It seems that the increasing co-occurrence of these genes and elements may lead to an increase in the resistance rate against carbapenems among *A. baumannii* isolates.

Biofilm formation is suspected of being one of the key pathogenic features of *A. baumannii*, particularly with device-related infections. In this study, 89.47% of isolates of *A. baumannii* were biofilm producers. Out of 16 isolates, 9 (47.37%) strong biofilm producers and 5 (21.05%) moderate biofilm producers were MDR. There was a statistically significant relationship between biofilm formation and antibiotic resistance. Similar results of 100%, 77% 100%, and 98.5%, respectively in India[62], South Korea[63,64], Algeria[65], and Egypt[66] showed a higher prevalence of biofilm formation.

The strong ability to form biofilms by *A. baumannii* is associated with a significant increase in antibiotic resistance of the bacteria as biofilms limit the diffusion of antibiotics to the site of action due to its components or alter the phenotypes or genotypes of the strains[67]. In this study, 84.21% of the *A. baumannii* strains were MDR, having the ability to form biofilms. This finding is in line with a previous study from Tehran where 92% biofilm-forming *A. baumannii* was reported to be MDR and 86% of isolates were extensively drug-resistance[68].

Impaired drug diffusion due to microbial aggregations, overexpression of the exopolymeric substance (EPS) matrix, alterations in microbial phenotypic and genotypic features due to stress responses, and physiological heterogeneity due to physicochemical gradients and persisters enhance antibiotic resistance in the biofilm phenotype[69]. Biofilm formation which is associated with long-term persistence in the hospital environment[64] depends on an interaction between three main components: the bacterial cells, the attachment surface, and the surrounding medium[65]. Thus, interventions such as contact precautions, environmental cleaning, active surveillance, and restrictions on administering broad-spectrum antibiotics can be used for controlling *A. baumannii*[64].

The study also evaluated the correlation between biofilm formation and resistance to carbapenem among isolates of *A. baumannii* and reported a significant statistical association. Although among carbapenem sensitive isolates, a strong biofilm producer was seen, the majority of carbapenem-resistance isolates were strong biofilm producers which explains some association exists between biofilm formation and antibiotic resistance among *A. baumannii* strains.

The ability of *A. baumannii* to attach and form biofilms in abiotic surfaces has increased the opportunistic infections in hospitalized patients with the increased antibiotic resistance rate. Other factors attributing to the increasing rate of *A. baumannii* infection in the hospital environment are inappropriate or inadequate management of infections, poor sanitation, inappropriate machinery handling practices by hospital personnel, misuse and

overuse of antibiotics, and lack of national guidelines and regulations for the use of antibiotics[66].

Hence, for reducing the incidence of antibiotic resistance, regular surveillance of hospital-associated infections, prescription of antibiotics based on antibiogram obtained from the microbiological analysis, and formulation of definitive antibiotic policy may be helpful. As biofilm production by *Acinetobacter* was associated with antibiotic resistance in this study, the screening tests for biofilm production in the laboratory may be useful to predict the drug resistance among the isolates. However, it is not clear whether any underlying mechanisms correlate biofilm production and antibiotic resistance. Therefore, future studies are recommended to understand the co-expression of biofilm and resistance-related genes. This study might be helpful to carry out future studies capable of covering a wider range of healthcare settings and community surveillance to establish the actual burden of disease and the prevalence rates of multidrug-resistant strains.

5. Conclusion

This study reported the carbapenemase-producing *A. baumannii* to be sensitive towards colistin and tetracycline, hence these are recommended as potential drugs to treat the infections caused by carbapenem resistance *Acinetobacter* species. In most of the isolates of multidrug-resistance *A. baumannii,* both $bla_{\text{OXA-51}}$ and $bla_{\text{OXA-23}}$ were detected as the determinant factors for carbapenem resistance having a direct relation with biofilm formation.

Author Contributions: UG is the primary author who has developed the study methodology, carried out laboratory investigations, and prepared the manuscript. The SS planned the experiments and guided the project. RK, SK, SKC, and MN contributed to the critical revision, data analysis, and editing of the manuscript. DRJ has contributed to the overall editing, review, and supervision of the project. The final version of the manuscript was read and accepted by all authors.

Funding: This work was funded by the University Grant Commission (UGC), Nepal through Master Thesis Grant Program (MRS-75/76-S &T-47).

Acknowledgments: We would like to acknowledge Central Department of Microbiology, Tribhuvan University, Phect-Nepal Kirtipur Hospital and all the staff of the research department for guiding the study. We would also like to thank University Grants Commission (UGC), Nepal for funding this project.

Conflicts of Interest: The authors declare no conflict of interest.

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