



Original Research Article

ASSOCIATION OF BIOFILM-FORMING CAPACITY WITH ANTIMICROBIAL RESISTANCE AND VIRULENCE PROFILES AMONG ACINETOBACTER BAUMANNII AND PSEUDOMONAS AERUGINOSA ISOLATED FROM DEVICE-ASSOCIATED INFECTIONS

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ABSTRACT

Background: Device-associated infections caused by *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are important healthcare-associated infections, particularly in critically ill patients. Biofilm formation contributes to persistence on medical devices and may be associated with antimicrobial resistance and enhanced virulence, thereby complicating treatment and infection control. **Aim:** To assess the association of biofilm-forming capacity with antimicrobial resistance and virulence profiles among *A. baumannii* and *P. aeruginosa* isolated from device-associated infections.

Materials and Methods: This hospital-based analytical cross-sectional study included 80 patients with device-associated infections at a tertiary care hospital. A total of 44 *A. baumannii* and 36 *P. aeruginosa* isolates were identified. Antimicrobial susceptibility testing was performed using standard methods, while biofilm formation was assessed by the microtiter plate crystal violet assay. Selected resistance and virulence genes were detected by polymerase chain reaction. Data were analyzed using IBM SPSS Statistics version 27.0, with $p < 0.05$ considered statistically significant.

Results: Biofilm formation was detected in 71 (88.75%) isolates, including 90.91% of *A. baumannii* and 86.11% of *P. aeruginosa*. Moderate-to-strong biofilm formation occurred in 53 (66.25%) isolates. Multidrug resistance was present in 55 (68.75%) isolates and was significantly higher in *A. baumannii* than *P. aeruginosa* (79.55% vs 55.56%; $p = 0.021$). MDR was significantly more frequent among moderate/strong biofilm producers than non/weak producers (83.02% vs 40.74%; OR=7.11; $p < 0.001$). Carbapenem resistance was also significantly associated with moderate-to-strong biofilm formation (75.47% vs 37.04%; $p = 0.001$). Among *A. baumannii*, *bap*, *csuE*, and *ompA* were significantly associated with stronger biofilm production, while *blaVIM* showed a significant association among *P. aeruginosa* isolates.

Conclusion: Biofilm formation was strongly associated with multidrug resistance, carbapenem resistance, and important virulence determinants. Routine assessment of biofilm-related characteristics may help improve antimicrobial stewardship and infection-control strategies for device-associated infections.

Keywords: *Acinetobacter baumannii*; *Pseudomonas aeruginosa*; Biofilm; Antimicrobial resistance; Device-associated infections.

INTRODUCTION

Device-associated infections constitute an important component of healthcare-associated infections and remain a major challenge in modern hospital practice. The increasing use of invasive medical devices, including endotracheal tubes, urinary catheters, central venous catheters, and other indwelling devices, has substantially improved the management of critically ill patients but simultaneously provides surfaces that facilitate microbial attachment, colonization, and persistence. Device-associated infections commonly manifest as ventilator-associated pneumonia, catheter-associated urinary tract infection, and central line-associated bloodstream infection. Patients admitted to intensive care units are particularly vulnerable because of prolonged hospitalization, severe underlying illnesses, repeated invasive procedures, and frequent exposure to broad-spectrum antimicrobial agents. An important microbiological feature of these infections is the capacity of microorganisms to adhere to artificial surfaces and develop structured biofilms, which may subsequently serve as persistent reservoirs of infection.¹ Among Gram-negative bacteria responsible for healthcare-associated infections, *Acinetobacter baumannii* has emerged as a particularly important opportunistic pathogen. It can survive for prolonged periods in the hospital environment and colonize both patients and healthcare-associated surfaces. The organism is associated with severe infections including hospital-acquired pneumonia, ventilator-associated pneumonia, bloodstream infections, urinary tract infections, wound infections, and sepsis. Its clinical importance is enhanced by a remarkable ability to acquire antimicrobial resistance determinants and adapt to adverse environmental conditions. Multiple mechanisms contribute to antimicrobial resistance in *A. baumannii*, including production of β -lactamases, alterations in membrane permeability, modification of antimicrobial targets, increased activity of efflux pumps, and acquisition of resistance genes through mobile genetic elements. In addition, several virulence determinants involving outer membrane proteins, capsular structures, iron acquisition systems, adherence mechanisms, and biofilm development facilitate its persistence and pathogenic potential.² *Pseudomonas aeruginosa* is another major opportunistic Gram-negative pathogen associated with serious healthcare-associated infections, particularly among critically ill, immunocompromised, mechanically ventilated, and device-dependent patients. Its ability to survive under diverse environmental conditions and its relatively large and adaptable genome provide considerable advantages in hospital environments. *P. aeruginosa* possesses both intrinsic and acquired mechanisms of antimicrobial resistance, including reduced outer-membrane permeability, active multidrug efflux systems, β -lactamase production,

alterations of antimicrobial targets, and acquisition of transferable resistance determinants. At the same time, its pathogenicity is supported by numerous virulence factors, including exotoxins, proteases, elastases, siderophores, pigments, secretion systems, motility structures, quorum-sensing mechanisms, and extracellular polysaccharides. These properties allow *P. aeruginosa* to establish both acute and persistent infections and complicate successful antimicrobial therapy.³ Biofilm formation represents a major survival and virulence strategy of *P. aeruginosa*. Biofilm development involves initial attachment to a surface, irreversible adherence, microcolony formation, maturation into a structured microbial community, and eventual dispersal of organisms to new sites. Within the mature biofilm, bacterial cells are embedded within a self-produced extracellular matrix composed of polysaccharides, extracellular DNA, proteins, and other macromolecules. Quorum-sensing networks regulate several aspects of biofilm development and coordinate expression of multiple virulence determinants. The biofilm environment protects bacterial cells against environmental stresses and host immune responses and facilitates long-term persistence on medical devices. Genes involved in alginate production and virulence, together with type III secretion-associated determinants such as *exoS* and *exoU*, contribute to the complex pathogenic behaviour of clinical *P. aeruginosa* isolates.⁴ Biofilm formation is also central to the persistence of *A. baumannii* on biotic and abiotic surfaces. The establishment and maturation of its biofilm involve multiple cellular structures and regulatory mechanisms. Biofilm-associated protein encoded by *bap* contributes to biofilm maturation and maintenance, while the *Csu* pili assembly system, including *csuE*, facilitates adherence to abiotic surfaces. Outer membrane protein A, encoded by *ompA*, has important roles in adhesion, biofilm formation, host-cell interaction, and pathogenicity. The ability of *A. baumannii* to combine environmental persistence with biofilm formation creates a particular challenge in healthcare settings where medical devices may provide favourable surfaces for prolonged bacterial colonization. Understanding these molecular determinants is therefore important for characterizing the virulence potential of clinical isolates.⁵ The relationship between biofilm formation and antimicrobial resistance is complex and clinically relevant. Bacteria within biofilms display physiological characteristics that differ substantially from free-living planktonic organisms. Restricted antimicrobial penetration, altered metabolic activity, nutrient limitation, heterogeneous microenvironments, stress responses, persister-cell formation, and biofilm-associated gene expression can collectively reduce antimicrobial effectiveness. In *P. aeruginosa*, biofilm growth may also create favourable conditions for the emergence and selection of resistant subpopulations during

persistent infection. Therefore, routine antimicrobial susceptibility patterns alone may not fully represent the capacity of an organism to persist on an infected medical device. Simultaneous assessment of antimicrobial resistance and biofilm-forming capacity may provide a more comprehensive understanding of the clinical behaviour of these pathogens.⁶

MATERIALS AND METHODS

This hospital-based analytical cross-sectional study was conducted in the Department of Microbiology at a tertiary care hospital. The study included 80 patients with clinically suspected device-associated infections from whom *Acinetobacter baumannii* or *Pseudomonas aeruginosa* was isolated. Device-associated infections included ventilator-associated pneumonia, catheter-associated urinary tract infection, and central line-associated bloodstream infection. The study was designed to determine the association between biofilm-forming capacity, antimicrobial resistance patterns, and virulence characteristics of the recovered isolates. A total of 80 patients fulfilling the diagnostic criteria for device-associated infections and yielding a significant, non-duplicate isolate of either *A. baumannii* or *P. aeruginosa* were included. Only one isolate per patient per infection episode was considered to avoid duplication. Relevant demographic and clinical information, including age, sex, hospital unit, underlying comorbidities, type of invasive device, duration of device use before infection, previous hospitalization, intensive care unit admission, and prior antimicrobial exposure, was recorded using a structured data collection form.

Inclusion and Exclusion Criteria

Patients of either sex with clinically and microbiologically confirmed device-associated infection caused by *A. baumannii* or *P. aeruginosa* were included. Patients with polymicrobial specimens in which the clinical significance of the target organism could not be established, duplicate isolates from the same infection episode, improperly collected or transported samples, isolates considered colonizers or contaminants, and patients with incomplete essential clinical information were excluded.

Methodology

Specimen Collection and Microbiological

Processing: Clinical specimens were collected aseptically according to the suspected device-associated infection. Endotracheal aspirates or other lower respiratory tract specimens were collected from patients with suspected ventilator-associated pneumonia, urine samples from catheterized patients with suspected catheter-associated urinary tract infection, and paired or appropriately collected blood cultures from patients with suspected central line-associated bloodstream infection. Specimens

were transported promptly to the microbiology laboratory and processed using standard microbiological techniques. Primary inoculation was performed on appropriate culture media, and isolates were identified on the basis of colony morphology, Gram staining, oxidase reaction, motility, biochemical characteristics, and other routine identification tests. Automated identification systems were used where available.

Antimicrobial Susceptibility Testing:

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar according to current Clinical and Laboratory Standards Institute recommendations. Antibiotics tested, as appropriate for the organism, included piperacillin-tazobactam, ceftazidime, cefepime, imipenem, meropenem, ciprofloxacin, levofloxacin, gentamicin, amikacin, and trimethoprim-sulfamethoxazole for *A. baumannii*. For *P. aeruginosa*, antipseudomonal β -lactams, carbapenems, fluoroquinolones, and aminoglycosides were evaluated. Minimum inhibitory concentrations for colistin and other selected agents were determined by broth microdilution where required. Isolates were categorized as susceptible, intermediate, or resistant according to recommended breakpoints. Multidrug-resistant isolates were identified according to internationally accepted definitions based on resistance to multiple antimicrobial categories.

Phenotypic Detection of Biofilm Formation:

Biofilm production was quantitatively assessed using the microtiter plate crystal violet assay. Overnight bacterial cultures were diluted in suitable broth medium and inoculated into sterile 96-well flat-bottom microtiter plates. After incubation, non-adherent cells were removed by washing, attached biofilms were fixed and stained with crystal violet, and excess stain was removed. The bound stain was solubilized and optical density was measured using a microplate reader. Each isolate was tested in triplicate, and appropriate positive and negative controls were included. Based on optical density values in relation to the calculated cut-off optical density, isolates were classified as non-biofilm producers, weak, moderate, or strong biofilm producers.

Assessment of Virulence Characteristics:

Virulence-associated phenotypic characteristics were evaluated using standardized laboratory methods. Parameters included hemolytic activity, protease production, lipase activity, motility, siderophore production, and surface-associated adherence characteristics wherever applicable. For *P. aeruginosa*, additional assessment included pyocyanin production and pigment formation, while for *A. baumannii*, surface motility and adherence-associated traits were documented. A cumulative virulence score was generated from the number of positive virulence characteristics demonstrated by each isolate to allow comparison with biofilm-forming ability and antimicrobial resistance.

Molecular Detection of Selected Resistance and Virulence Genes: Where molecular facilities were available, bacterial DNA was extracted using a standardized extraction procedure and conventional polymerase chain reaction was performed for selected clinically important genes. *A. baumannii* isolates were evaluated for representative resistance determinants such as **blaOXA-23-like**, **blaNDM**, and virulence or biofilm-associated genes such as **bap**, **csuE**, and **ompA**. *P. aeruginosa* isolates were screened for selected carbapenemase genes including **blaVIM**, **blaIMP**, and **blaNDM**, together with virulence-associated genes such as **lasB**, **algD**, **toxA**, **exoS**, and **exoU**. Positive and negative controls were included in each PCR run.

Quality Control: All culture media, biochemical tests, antimicrobial susceptibility procedures, biofilm assays, and molecular tests were subjected to routine quality-control procedures. Standard reference strains, including *Pseudomonas aeruginosa* ATCC 27853, were used for antimicrobial susceptibility quality control. Biofilm experiments were performed in replicates to minimize analytical variability.

Outcome Measures: The primary outcome was the association between the degree of biofilm formation and antimicrobial resistance. Secondary outcomes included the relationship of biofilm strength with multidrug-resistant phenotype, individual antimicrobial resistance patterns, virulence characteristics, resistance genes, virulence genes, type of device-associated infection, intensive care unit admission, and relevant clinical characteristics.

Statistical Analysis
Data were entered into Microsoft Excel and analyzed using IBM SPSS Statistics version 27.0. Continuous variables were expressed as mean $\pm$ standard deviation or median with interquartile range according to data distribution, whereas categorical variables were presented as frequencies and percentages. The Shapiro-Wilk test was used to assess normality. Categorical variables were compared using the chi-square test or Fisher's exact test. Continuous variables were compared using the independent-samples t-test, Mann-Whitney U test, one-way ANOVA, or Kruskal-Wallis test, as appropriate. Spearman or Pearson correlation was used to evaluate relationships between quantitative biofilm measurements and selected resistance or virulence parameters. Binary or multinomial logistic regression analysis was performed to identify independent predictors of moderate-to-strong biofilm formation and multidrug resistance. A *p* value <0.05 was considered statistically significant.

RESULTS

Table 1. Demographic and Clinical Characteristics According to Isolated Organism

A total of 80 patients with device-associated infections were included in the study, of whom 44

(55.00%) had *Acinetobacter baumannii* and 36 (45.00%) had *Pseudomonas aeruginosa* isolates. The overall mean age of the study population was 52.66 ± 16.34 years. The mean age of patients with *A. baumannii* infection was 54.10 ± 15.70 years, compared with 50.90 ± 17.10 years among those with *P. aeruginosa* infection. The difference in mean age between the two groups was not statistically significant ($p=0.391$). Among the total study population, 49 (61.25%) patients were male and 31 (38.75%) were female. Males constituted 29 (65.91%) of the *A. baumannii* group and 20 (55.56%) of the *P. aeruginosa* group, while females accounted for 15 (34.09%) and 16 (44.44%), respectively. The difference in sex distribution between the groups was not statistically significant ($p=0.344$). ICU admission was recorded in 57 (71.25%) patients overall. Among patients with *A. baumannii*, 34 (77.27%) required ICU admission, compared with 23 (63.89%) patients with *P. aeruginosa*. This difference was not statistically significant ($p=0.188$). Previous antimicrobial exposure was present in 47 (58.75%) patients, comprising 29 (65.91%) patients in the *A. baumannii* group and 18 (50.00%) patients in the *P. aeruginosa* group, with no statistically significant difference ($p=0.150$). Ventilator-associated pneumonia was the most frequent device-associated infection, occurring in 36 (45.00%) patients. It was observed in 24 (54.55%) patients with *A. baumannii* and 12 (33.33%) patients with *P. aeruginosa*. Catheter-associated urinary tract infection was present in 26 (32.50%) patients, including 12 (27.27%) in the *A. baumannii* group and 14 (38.89%) in the *P. aeruginosa* group. Central line-associated bloodstream infection was identified in 18 (22.50%) patients, comprising 8 (18.18%) with *A. baumannii* and 10 (27.78%) with *P. aeruginosa*. The overall distribution of device-associated infection types between the two organisms did not differ significantly ($p=0.164$).

Table 2. Distribution of Biofilm-Forming Capacity Among the Bacterial Isolates

Biofilm formation was detected in 71 of the 80 isolates, giving an overall biofilm positivity rate of 88.75%. Among *A. baumannii* isolates, 40 of 44 (90.91%) demonstrated some degree of biofilm formation, while 31 of 36 (86.11%) *P. aeruginosa* isolates were biofilm producers. The difference in overall biofilm production between the two organisms was not statistically significant ($p=0.503$). Among the 44 *A. baumannii* isolates, 4 (9.09%) were non-biofilm producers, 8 (18.18%) were weak biofilm producers, 15 (34.09%) showed moderate biofilm production, and 17 (38.64%) were strong biofilm producers. Among the 36 *P. aeruginosa* isolates, 5 (13.89%) were non-biofilm producers, 10 (27.78%) were weak producers, 13 (36.11%) were moderate producers, and 8 (22.22%) were strong biofilm producers. Overall, 9 (11.25%) isolates were non-biofilm producers, 18 (22.50%) were weak producers, 28 (35.00%) were moderate producers,

and 25 (31.25%) were strong producers. The distribution of isolates across the four biofilm categories was not statistically significant between the two organisms ($p=0.400$). Moderate-to-strong biofilm formation was observed in 53 (66.25%) isolates overall. This included 32 of 44 (72.73%) *A. baumannii* isolates and 21 of 36 (58.33%) *P. aeruginosa* isolates. Although moderate-to-strong biofilm formation was numerically higher among *A. baumannii* isolates, the difference was not statistically significant ($p=0.264$).

Table 3. Antimicrobial Resistance Profile of *A. baumannii* and *P. aeruginosa* Isolates

A high level of antimicrobial resistance was observed among both bacterial species. Resistance to piperacillin-tazobactam was found in 49 (61.25%) isolates overall, including 31 (70.45%) *A. baumannii* and 18 (50.00%) *P. aeruginosa* isolates. The difference approached but did not reach statistical significance ($p=0.062$). Ceftazidime resistance was present in 55 (68.75%) isolates, including 33 (75.00%) *A. baumannii* and 22 (61.11%) *P. aeruginosa* isolates ($p=0.182$). Cefepime resistance was recorded in 52 (65.00%) isolates, consisting of 32 (72.73%) *A. baumannii* and 20 (55.56%) *P. aeruginosa* isolates ($p=0.109$). Resistance to imipenem was documented in 48 (60.00%) isolates overall. Imipenem resistance was significantly higher among *A. baumannii* isolates, where 31 (70.45%) were resistant, compared with 17 (47.22%) *P. aeruginosa* isolates ($p=0.035$). Similarly, meropenem resistance was found in 50 (62.50%) isolates overall, including 32 (72.73%) *A. baumannii* and 18 (50.00%) *P. aeruginosa* isolates. This difference was also statistically significant ($p=0.037$). Ciprofloxacin resistance was identified in 50 (62.50%) isolates, comprising 31 (70.45%) *A. baumannii* and 19 (52.78%) *P. aeruginosa* isolates ($p=0.104$). Levofloxacin resistance was found in 47 (58.75%) isolates, including 29 (65.91%) *A. baumannii* and 18 (50.00%) *P. aeruginosa* isolates ($p=0.150$). Gentamicin resistance was detected in 42 (52.50%) isolates, including 26 (59.09%) *A. baumannii* and 16 (44.44%) *P. aeruginosa* isolates ($p=0.192$). Amikacin resistance was present in 36 (45.00%) isolates, comprising 23 (52.27%) *A. baumannii* and 13 (36.11%) *P. aeruginosa* isolates ($p=0.148$). Colistin resistance was uncommon and was observed in only 3 (3.75%) isolates overall. This included 2 (4.55%) *A. baumannii* isolates and 1 (2.78%) *P. aeruginosa* isolate, with no statistically significant difference ($p=1.000$). Multidrug resistance was identified in 55 (68.75%) isolates overall. MDR was significantly more frequent among *A. baumannii*, affecting 35 (79.55%) isolates, compared with 20 (55.56%) *P. aeruginosa* isolates ($p=0.021$).

Table 4. Association of Moderate-to-Strong Biofilm Formation With Antimicrobial Resistance and Clinical Characteristics

Among the 80 isolates, 27 were classified as non-biofilm or weak biofilm producers, while 53

demonstrated moderate-to-strong biofilm formation. Multidrug resistance was observed in 11 of 27 (40.74%) non/weak biofilm-forming isolates compared with 44 of 53 (83.02%) moderate/strong biofilm-forming isolates. Moderate-to-strong biofilm-producing isolates had higher odds of multidrug resistance, with an odds ratio of 7.11 (95% CI: 2.49–20.33), and the association was statistically significant ($p<0.001$). Carbapenem resistance was identified in 10 (37.04%) non/weak biofilm producers and 40 (75.47%) moderate/strong biofilm producers. The odds of carbapenem resistance were 5.23 times higher among moderate-to-strong biofilm-forming isolates compared with non/weak biofilm producers (95% CI: 1.92–14.23), and the association was statistically significant ($p=0.001$). Resistance to five or more antimicrobial agents was observed in 8 (29.63%) non/weak biofilm-forming isolates compared with 37 (69.81%) moderate/strong biofilm-forming isolates. Moderate-to-strong biofilm production was associated with 5.49-fold higher odds of resistance to five or more antimicrobial agents (95% CI: 1.99–15.13), with a statistically significant association ($p=0.001$). Previous antimicrobial exposure was recorded in 12 (44.44%) patients infected with non/weak biofilm-forming isolates and 35 (66.04%) patients infected with moderate/strong biofilm-forming isolates. The corresponding odds ratio was 2.43 (95% CI: 0.94–6.27); however, this association did not reach statistical significance ($p=0.064$). ICU admission was documented in 14 (51.85%) patients with non/weak biofilm-producing isolates compared with 43 (81.13%) patients with moderate/strong biofilm-producing isolates. The odds of ICU admission were 3.99 times higher in the moderate/strong biofilm group (95% CI: 1.44–11.09), and the association was statistically significant ($p=0.006$).

Table 5. Association of Biofilm Formation With Virulence and Resistance Gene Profiles

Among the 44 *A. baumannii* isolates, 12 were categorized as non/weak biofilm producers and 32 as moderate/strong biofilm producers. The *blaOXA-23-like* gene was detected in 29 (65.91%) *A. baumannii* isolates overall. It was present in 5 of 12 (41.67%) non/weak biofilm producers and 24 of 32 (75.00%) moderate/strong biofilm producers. The difference did not reach statistical significance ($p=0.071$). The *blaNDM* gene was identified in 10 (22.73%) isolates overall, including 1 (8.33%) non/weak biofilm producer and 9 (28.13%) moderate/strong biofilm producers, with no statistically significant association ($p=0.241$). The biofilm-associated *bap* gene was detected in 28 (63.64%) *A. baumannii* isolates. It was present in 3 (25.00%) non/weak biofilm-forming isolates compared with 25 (78.13%) moderate/strong biofilm-forming isolates, demonstrating a statistically significant association ($p=0.003$). The *csuE* gene was detected in 33 (75.00%) isolates overall and was present in 5 (41.67%) non/weak

producers compared with 28 (87.50%) moderate/strong producers, with a statistically significant difference ($p=0.004$). The *ompA* gene was the most frequently detected *A. baumannii* virulence-associated gene, being present in 38 (86.36%) isolates. It was detected in 8 (66.67%) non/weak biofilm producers and 30 (93.75%) moderate/strong biofilm producers, with a statistically significant association ($p=0.039$). Among the 36 *P. aeruginosa* isolates, 15 were non/weak biofilm producers and 21 were moderate/strong biofilm producers. The *bla_{VM}* gene was detected in 12 (33.33%) isolates overall. It was present in 2 (13.33%) non/weak biofilm producers and 10 (47.62%) moderate/strong biofilm producers. This difference was statistically significant ($p=0.040$). The *bla_{IMP}* gene was identified in 8 (22.22%) isolates, comprising 2 (13.33%) non/weak and 6 (28.57%) moderate/strong biofilm producers, with no significant association ($p=0.424$). The *bla_{NDM}* gene was detected in 5

(13.89%) isolates, including 1 (6.67%) non/weak and 4 (19.05%) moderate/strong biofilm producers, and the difference was not statistically significant ($p=0.376$). Among the virulence-associated genes of *P. aeruginosa*, *lasB* was detected in 27 (75.00%) isolates, comprising 9 (60.00%) non/weak and 18 (85.71%) moderate/strong biofilm producers ($p=0.122$). The *algD* gene was identified in 29 (80.56%) isolates overall, including 10 (66.67%) non/weak and 19 (90.48%) moderate/strong biofilm producers ($p=0.103$). The *toxA* gene was found in 23 (63.89%) isolates, including 7 (46.67%) non/weak and 16 (76.19%) moderate/strong biofilm producers ($p=0.090$). The *exoS* gene was detected in 20 (55.56%) isolates, comprising 7 (46.67%) non/weak and 13 (61.90%) moderate/strong biofilm producers ($p=0.500$). The *exoU* gene was found in 11 (30.56%) isolates, including 3 (20.00%) non/weak and 8 (38.10%) moderate/strong biofilm producers ($p=0.295$).

Table 1: Demographic and Clinical Characteristics According to Isolated Organism

Parameter	<i>A. baumannii</i> (n=44)	<i>P. aeruginosa</i> (n=36)	Total (n=80)	P value
Age, years, Mean $\pm$ SD	54.10 $\pm$ 15.70	50.90 $\pm$ 17.10	52.66 $\pm$ 16.34	0.391
Male	29 (65.91%)	20 (55.56%)	49 (61.25%)	0.344
Female	15 (34.09%)	16 (44.44%)	31 (38.75%)	
ICU admission	34 (77.27%)	23 (63.89%)	57 (71.25%)	0.188
Previous antimicrobial exposure	29 (65.91%)	18 (50.00%)	47 (58.75%)	0.150
Ventilator-associated pneumonia	24 (54.55%)	12 (33.33%)	36 (45.00%)	0.164
Catheter-associated urinary tract infection	12 (27.27%)	14 (38.89%)	26 (32.50%)	
Central line-associated bloodstream infection	8 (18.18%)	10 (27.78%)	18 (22.50%)	

P value for device-associated infection represents comparison of the overall distribution of the three infection types.

Table 2: Distribution of Biofilm-Forming Capacity Among the Bacterial Isolates

Biofilm-forming capacity	<i>A. baumannii</i> (n=44)	<i>P. aeruginosa</i> (n=36)	Total (n=80)	P value
Non-biofilm producer	4 (9.09%)	5 (13.89%)	9 (11.25%)	0.400
Weak biofilm producer	8 (18.18%)	10 (27.78%)	18 (22.50%)	
Moderate biofilm producer	15 (34.09%)	13 (36.11%)	28 (35.00%)	
Strong biofilm producer	17 (38.64%)	8 (22.22%)	25 (31.25%)	
Any biofilm production	40 (90.91%)	31 (86.11%)	71 (88.75%)	0.503
Moderate-to-strong biofilm formation	32 (72.73%)	21 (58.33%)	53 (66.25%)	0.264

Table 3: Antimicrobial Resistance Profile of *A. baumannii* and *P. aeruginosa* Isolates

Antimicrobial agent/resistance pattern	<i>A. baumannii</i> (n=44)	<i>P. aeruginosa</i> (n=36)	Total (n=80)	P value
Piperacillin-tazobactam resistant	31 (70.45%)	18 (50.00%)	49 (61.25%)	0.062
Ceftazidime resistant	33 (75.00%)	22 (61.11%)	55 (68.75%)	0.182
Cefepime resistant	32 (72.73%)	20 (55.56%)	52 (65.00%)	0.109
Imipenem resistant	31 (70.45%)	17 (47.22%)	48 (60.00%)	0.035
Meropenem resistant	32 (72.73%)	18 (50.00%)	50 (62.50%)	0.037
Ciprofloxacin resistant	31 (70.45%)	19 (52.78%)	50 (62.50%)	0.104
Levofloxacin resistant	29 (65.91%)	18 (50.00%)	47 (58.75%)	0.150
Gentamicin resistant	26 (59.09%)	16 (44.44%)	42 (52.50%)	0.192
Amikacin resistant	23 (52.27%)	13 (36.11%)	36 (45.00%)	0.148
Colistin resistant	2 (4.55%)	1 (2.78%)	3 (3.75%)	1.000
Multidrug-resistant (MDR)	35 (79.55%)	20 (55.56%)	55 (68.75%)	0.021

Table 4: Association of Moderate-to-Strong Biofilm Formation with Antimicrobial Resistance and Clinical Characteristics

Parameter	Non/weak biofilm (n=27)	Moderate/strong biofilm (n=53)	Odds Ratio (95% CI)	P value
Multidrug resistance	11 (40.74%)	44 (83.02%)	7.11 (2.49–20.33)	<0.001
Carbapenem resistance	10 (37.04%)	40 (75.47%)	5.23 (1.92–14.23)	0.001
Resistance to ≥ 5 antimicrobial agents	8 (29.63%)	37 (69.81%)	5.49 (1.99–15.13)	0.001

Previous antimicrobial exposure	12 (44.44%)	35 (66.04%)	2.43 (0.94–6.27)	0.064
ICU admission	14 (51.85%)	43 (81.13%)	3.99 (1.44–11.09)	0.006

Table 5: Association of Biofilm Formation with Virulence and Resistance Gene Profiles

Organism/marker	Non/weak biofilm n (%)	Moderate/strong biofilm n (%)	Total positive n (%)	P value
A. baumannii (n=44)	n=12	n=32		
blaOXA-23-like	5 (41.67%)	24 (75.00%)	29 (65.91%)	0.071
blaNDM	1 (8.33%)	9 (28.13%)	10 (22.73%)	0.241
bap	3 (25.00%)	25 (78.13%)	28 (63.64%)	0.003
csuE	5 (41.67%)	28 (87.50%)	33 (75.00%)	0.004
ompA	8 (66.67%)	30 (93.75%)	38 (86.36%)	0.039
P. aeruginosa (n=36)	n=15	n=21		
blaVIM	2 (13.33%)	10 (47.62%)	12 (33.33%)	0.040
blaIMP	2 (13.33%)	6 (28.57%)	8 (22.22%)	0.424
blaNDM	1 (6.67%)	4 (19.05%)	5 (13.89%)	0.376
lasB	9 (60.00%)	18 (85.71%)	27 (75.00%)	0.122
algD	10 (66.67%)	19 (90.48%)	29 (80.56%)	0.103
toxA	7 (46.67%)	16 (76.19%)	23 (63.89%)	0.090
exoS	7 (46.67%)	13 (61.90%)	20 (55.56%)	0.500
exoU	3 (20.00%)	8 (38.10%)	11 (30.56%)	0.295

DISCUSSION

The present study included 80 patients with device-associated infections, with *Acinetobacter baumannii* accounting for 44 (55.00%) isolates and *Pseudomonas aeruginosa* for 36 (45.00%). The overall mean age was 52.66 ± 16.34 years, and males constituted 61.25% of the patients. ICU admission was required in 71.25% of patients and was more frequent among those infected with *A. baumannii* (77.27%) than *P. aeruginosa* (63.89%), although the difference was not statistically significant ($p=0.188$). Ventilator-associated pneumonia was the most frequent device-associated infection (45.00%), followed by catheter-associated urinary tract infection (32.50%) and central line-associated bloodstream infection (22.50%). Al-Shamiri et al. (2021) studied 70 clinical *A. baumannii* isolates and reported that 54 (77.10%) originated from ICU patients, which closely corresponded with the 77.27% ICU admission observed among *A. baumannii*-infected patients in the present study. They additionally reported MDR and XDR frequencies of 55.70% and 31.40%, respectively. The close agreement in the ICU-associated proportion emphasizes the predominance of *A. baumannii* among critically ill hospitalized patients requiring invasive devices.⁷ Biofilm production represented a prominent characteristic of the isolates in the present study. Overall, 71 of 80 (88.75%) isolates demonstrated biofilm formation, including 40 of 44 (90.91%) *A. baumannii* and 31 of 36 (86.11%) *P. aeruginosa* isolates. Among *A. baumannii*, 18.18% were weak, 34.09% moderate, and 38.64% strong biofilm producers, while 9.09% did not produce biofilm. Yang et al. (2019), in their analysis of 154 clinical *A. baumannii* isolates, reported biofilm formation in 93.50% of isolates, with 15.60% classified as weak, 32.50% as moderate, and 45.40% as strong biofilm producers. Their moderate biofilm rate of 32.50% was particularly close to the 34.09% observed in the present study, while their strong biofilm rate of

45.40% was somewhat higher than the present value of 38.64%. Thus, both studies demonstrated that the great majority of clinical *A. baumannii* isolates were capable of biofilm formation, with moderate-to-strong production constituting the predominant phenotype.^[8] Among *P. aeruginosa* isolates in the present study, 86.11% produced biofilm, comprising 27.78% weak, 36.11% moderate, and 22.22% strong biofilm producers. Moderate-to-strong biofilm formation was observed in 58.33% of *P. aeruginosa* isolates, while 55.56% demonstrated an MDR phenotype. Abdulhaq et al. (2020) evaluated 52 clinical *P. aeruginosa* isolates and demonstrated biofilm production by the microtiter plate assay in 49 (94.23%) isolates, a proportion higher than the 86.11% observed in the present study. They identified MDR in 20 (38.46%) isolates, compared with a higher MDR prevalence of 55.56% in the present *P. aeruginosa* isolates. Importantly, all MDR isolates in their study demonstrated biofilm production, supporting the observed relationship between biofilm formation and antimicrobial resistance. Differences in the absolute proportions may reflect differences in specimen distribution, hospital antimicrobial pressure, patient population, and circulating bacterial clones.^[9] Considerable antimicrobial resistance was observed, particularly among *A. baumannii*. Resistance among *A. baumannii* isolates was 75.00% to ceftazidime, 72.73% to cefepime, 70.45% to imipenem, 72.73% to meropenem, 70.45% to ciprofloxacin, 65.91% to levofloxacin, 59.09% to gentamicin and 52.27% to amikacin. Carbapenem resistance was significantly higher in *A. baumannii* than *P. aeruginosa* for both imipenem (70.45% vs 47.22%; $p=0.035$) and meropenem (72.73% vs 50.00%; $p=0.037$). Shenkutie et al. (2020) reported resistance among their *A. baumannii* isolates of 60.60% to ceftazidime, 51.90% to imipenem, 51.90% to meropenem, 51.90% to ciprofloxacin, and 47.10% to gentamicin. These values were generally lower than the corresponding 75.00%, 70.45%, 72.73%, 70.45%, and 59.09% observed in the present study.

Conversely, their reported colistin resistance of 20.20% was substantially higher than the 4.55% observed among *A. baumannii* isolates in the present study.^[10] Multidrug resistance was documented in 55 (68.75%) isolates overall and was significantly more common among *A. baumannii* than *P. aeruginosa* (79.55% vs 55.56%; $p=0.021$). A particularly important finding was the relationship between the magnitude of biofilm production and resistance. MDR was present in 83.02% of moderate/strong biofilm producers compared with only 40.74% of non/weak producers, corresponding to an odds ratio of 7.11 (95% CI: 2.49–20.33; $p<0.001$). Similarly, carbapenem resistance occurred in 75.47% versus 37.04% (OR=5.23; $p=0.001$), while resistance to five or more antimicrobial agents occurred in 69.81% versus 29.63% (OR=5.49; $p=0.001$). Gautam et al. (2023) reported an MDR prevalence of 78.82% among 307 *A. baumannii* isolates, which was remarkably similar to the 79.55% MDR rate among *A. baumannii* in the present study. They detected biofilm in 66.78% of isolates and found that 71.07% of MDR isolates were biofilm producers, with a significant association between MDR and biofilm production. Their findings support the present observation that increasing biofilm capacity occurred alongside a substantially greater frequency of multidrug resistance.^[11] Molecular analysis of *A. baumannii* demonstrated that blaOXA-23-like and blaNDM were present in 65.91% and 22.73% of isolates, respectively, while the biofilm/virulence-associated genes *bap*, *csuE*, and *ompA* occurred in 63.64%, 75.00%, and 86.36%. The *bap* gene was significantly more common among moderate/strong than non/weak biofilm producers (78.13% vs 25.00%; $p=0.003$). Similar associations were demonstrated for *csuE* (87.50% vs 41.67%; $p=0.004$) and *ompA* (93.75% vs 66.67%; $p=0.039$). Khoshnood et al. (2023) evaluated 50 *A. baumannii* isolates and reported *ompA* and *csuE* in 86.00% each. Their *ompA* prevalence was almost identical to the 86.36% recorded in the present study, whereas their *csuE* prevalence of 86.00% exceeded the present overall prevalence of 75.00%. They also reported 52.00% strong, 36.00% moderate, and 12.00% weak biofilm producers and demonstrated a significant relationship between strong biofilm production and the highly resistant phenotype. These molecular findings are comparable with the present significant concentration of *bap*, *csuE*, and *ompA* among moderate-to-strong biofilm-producing isolates.^[12] Among the 36 *P. aeruginosa* isolates, carbapenemase genes blaVIM, blaIMP, and blaNDM were detected in 33.33%, 22.22%, and 13.89%, respectively. Of particular importance, blaVIM occurred in 47.62% of moderate/strong biofilm producers compared with only 13.33% of non/weak producers ($p=0.040$). Bogiel et al. (2021) investigated 107 non-duplicate carbapenem-resistant *P. aeruginosa* isolates and detected blaVIM in 32 (29.90%) isolates, which was close to the overall

blaVIM prevalence of 33.33% in the present study. In contrast, none of their isolates carried blaIMP, whereas blaIMP was detected in 22.22% of the present isolates. Bogiel et al. also demonstrated extensive carriage of virulence determinants among carbapenem-resistant isolates, including algD in 92.50%, *toxA* in 96.30%, and *exoS* in 58.90%. The present findings therefore demonstrate a similarly substantial coexistence of acquired carbapenem-resistance determinants and virulence-associated characteristics among clinical *P. aeruginosa* isolates.^[13] The virulence profile of *P. aeruginosa* further demonstrated high frequencies of algD (80.56%), *lasB* (75.00%), *toxA* (63.89%), *exoS* (55.56%), and *exoU* (30.56%). Each of these genes was numerically more frequent among moderate/strong biofilm producers; algD was detected in 90.48% versus 66.67%, *lasB* in 85.71% versus 60.00%, *toxA* in 76.19% versus 46.67%, *exoS* in 61.90% versus 46.67%, and *exoU* in 38.10% versus 20.00% of moderate/strong and non/weak biofilm producers, respectively, although these differences did not reach statistical significance. Farhan et al. (2023) evaluated 126 clinical *P. aeruginosa* isolates and identified biofilm production in 80.00%, including 18.00% strong, 34.00% moderate and 28.00% weak producers. Among their biofilm-producing isolates, algD, *toxA*, and *exoS* were reported in 96.00%, 80.00%, and 74.00%, respectively. These frequencies were higher than the corresponding overall values of 80.56%, 63.89%, and 55.56% in the present study, although the frequencies among the moderate/strong biofilm group in the present study—90.48%, 76.19%, and 61.90%—showed the same pattern of frequent virulence-gene carriage among isolates with greater biofilm-forming capacity.^[14]

CONCLUSION

The study demonstrated a high prevalence of biofilm formation and multidrug resistance among *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolated from device-associated infections. Moderate-to-strong biofilm production was significantly associated with multidrug resistance, carbapenem resistance, and resistance to multiple antimicrobial agents. Biofilm-associated and virulence genes such as *bap*, *csuE*, *ompA*, and blaVIM were more frequently detected among stronger biofilm producers. These findings highlight the close relationship between biofilm formation, antimicrobial resistance, and virulence and emphasize the need for strict infection-control measures and appropriate antimicrobial stewardship.

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