



# Epidemiology of Biofilm Producing *Acinetobacter baumannii* Nosocomial Isolates from a Tertiary Care Hospital in Egypt: A Cross-Sectional Study

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**Objective:** This cross-sectional study aims to determine the prevalence and associated risk factors of biofilm-producing *A. baumannii* nosocomial isolates from a tertiary care hospital, as well as to investigate any possible association of biofilm formation with the distribution of biofilm-related genotypes and antibiotic resistance phenotypes.

**Methods:** A total of 94 non-duplicate *A. baumannii* nosocomial isolates were identified, their biofilm formation was quantitatively detected using the modified microtiter plate assay, and their susceptibilities to different antibiotics were determined using the breakpoint method. Isolates were then subjected to PCR assays targeting *bap*, *ompA* and *bla<sub>PER-1</sub>* genes.

**Results:** The majority (70.1%) of isolates were biofilm producers. The most prevalent biofilm gene was *ompA* (63.8%), followed by *bap* (13.8%) and *bla<sub>PER-1</sub>* (10.6%). The presence of multi- and extensive-drug resistance (MDR and XDR) was significantly associated with biofilm producers ( $p = 0.017$  and  $0.002$ , respectively). The length of hospital stay ( $aOR = 0.023$ ), the presence of *ompA* gene ( $aOR = 0.286$ ) or *bap* gene ( $aOR = 0.346$ ), ampicillin/sulbactam resistance ( $aOR = 1$ ), and the presence of MDR ( $aOR = -0.329$ ) or XDR ( $aOR = -0.252$ ) were considered significant risk factors associated with biofilm-producing isolates.

**Conclusion:** The high prevalence of biofilm-producing MDR and XDR nosocomial isolates in this study is worrisome and alarming. Characterization of risk factors could help control the continuous selection and transfer of this serious *A. baumannii* phenotype inside hospitals and improve the quality of patients' care.

**Keywords:** *Acinetobacter*, biofilm, MDR, XDR, nosocomial infection

## Introduction

*A. baumannii* is still considered a serious nosocomial pathogen as it accounts for a wide range of healthcare-associated infections, including bacteraemia, urinary tract infection, secondary meningitis, skin and soft tissues infection, and nosocomial and ventilator-associated pneumonia, especially in patients admitted to intensive care and burn units.<sup>1–3</sup> These infections are usually associated with a high mortality rate of up to 26% for hospitalized patients<sup>4</sup> to up to 43% for intensive care unit (ICU) patients.<sup>5</sup> *A. baumannii* is the first in the critical priority list of pathogens that poses the greatest threat to human health according to the World Health Organization.<sup>6</sup> In the last few years, the scenario is more dramatic with a continuous surge of multi-, extensive- and pan-drug resistant (MDR, XDR, and PDR) *A. baumannii* nosocomial isolates, some of which are even resistant to tigecycline and colistin, the last resort drugs in therapeutic protocols.

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Worldwide, the successful model of *A. baumannii* as an endemic pathogen in healthcare facilities has been attributed to several factors, such as its remarkable intrinsic and acquired resistance to multiple antimicrobial classes, including penicillins, extended-spectrum cephalosporins, fluoroquinolones and carbapenems,<sup>1,2</sup> its ability to produce biofilm and persist on biotic and abiotic surfaces such as environmental surfaces and medical devices,<sup>3</sup> and its high intake ability of foreign genetic elements to survive under harsh conditions and antibiotic treatment.<sup>1,7</sup>

The ability of *A. baumannii* to form biofilm is primarily considered an effective strategy to increase the bacterial survival, adherence to mucosal surfaces, dormancy in deep biofilm layers, and persistence in hospital environment under stress conditions.<sup>1,7,8</sup> Several virulence factors are involved in the biofilm formation of *A. baumannii* such as the biofilm-associated protein (Bap) encoded by the *bap* gene, the outer membrane protein A (OmpA) encoded by the *ompA* gene,<sup>9</sup> chaperon-usher pilus (Csu), extracellular exopolysaccharide (EPS), two-component system (BfmS/BfmR), poly- $\beta$ -(1,6)-N-acetyl glucosamine (PNAG) and quorum sensing system.<sup>8–11</sup> Besides, Lee et al<sup>7</sup> have shown that the ability of clinical isolates of *A. baumannii* to form biofilm and to adhere to respiratory epithelial cells is enhanced by the presence and expression of the *bla*<sub>PER-1</sub> gene.

Providing a new insight into the better understanding of the possible association between biofilm formation of *A. baumannii* nosocomial isolates and their antibiotic resistance phenotypes could be helpful for improving infection control procedures in healthcare facilities. However, the findings of previous clinical and epidemiological studies are still scarce and controversial.<sup>8–16</sup> Therefore, this study aims to determine the prevalence and associated risk factors of biofilm-producing *A. baumannii* nosocomial isolates from an Egyptian tertiary care hospital, as well as to investigate any possible association of biofilm formation with the distribution of biofilm-related genotypes and antibiotic resistance phenotypes.

## Materials and Methods

This cross-sectional observational study was conducted from August 2018 to July 2019 at the Menoufia University Hospitals (MUHs), a 760-bed tertiary care hospital in Shebeen El-Koum, a city in Egypt. The study was conducted according to the international guidelines of Strengthening the Reporting for Observational Studies in Epidemiology

(STROBE).<sup>17</sup> During the study period, all *A. baumannii* nosocomial isolates were collected from adult patients (one isolate from each patient) who were hospitalized for  $\geq 48$  hours and classified according to the Centres for Disease Control and Prevention/National Healthcare Safety Network (CDC/NHSN) criteria.<sup>18</sup>

## Demographic and Medical Details

Demographic and medical data of the hospitalized patients were obtained using a questionnaire, which included age, gender, type of (system involved for) infection (urinary tract infection, respiratory tract infection, skin and soft tissue infection, and primary bacteremia), ward admission (medical, surgical, burn unit and ICU), presence of comorbid conditions such as diabetes mellitus, hypertension, chronic pulmonary disease, and chronic heart disease, use of invasive procedures (central or peripheral venous catheter, urinary catheter and ventilator), length of stay, and history of previous antibiotic therapy.

## Laboratory Processing

The clinical specimens were collected from all patients and were sent to the microbiology laboratory department of the NLI in Menoufia for further processing. All *A. baumannii* isolates were identified using conventional biochemical tests,<sup>19</sup> the API 20NE (bioMérieux, Marcy-l'Etoile, France) and the VITEK 2 compact system (AST-GN90 susceptibility cards, bioMérieux), according to the manufacturers' instructions. Antimicrobial susceptibility testing was performed with the broth microdilution method for determination of the minimum inhibitory concentration (MIC) breakpoints, using the VITEK 2 compact system, and the E-test minimum inhibitory concentration method, using E-test strips (AB Biodisk, Solna, Sweden) on Mueller Hinton agar plates using Clinical and Laboratory Standards Institute (CLSI) guidelines.<sup>19</sup> The antibiotics tested included ceftazidime, cefotaxime, cefepime, ampicillin/sulbactam, piperacillin/tazobactam, piperacillin, amikacin, gentamycin, tobramycin, ciprofloxacin, gatifloxacin, trimethoprim/sulfamethoxazole, imipenem, meropenem, tigecycline, and colistin. *Pseudomonas aeruginosa* ATCC 27,853 and *Escherichia coli* ATCC 25,922 were used as quality control during antibiotic resistance testing.<sup>20</sup> To determine the susceptibility to antimicrobial agents, MIC breakpoints from the CLSI guidelines<sup>20</sup> were used as interpretative standards except for the tigecycline breakpoints, which were obtained from the US Food and Drug Administration (FDA).<sup>21</sup> The MDR was defined as resistance to  $\geq 3$  antimicrobial classes

(cephalosporins,  $\beta$ -lactam/ $\beta$ -lactamase inhibitor combinations, aminoglycosides, antipseudomonal carbapenems, and fluoroquinolones), and the XDR as resistance to all antibiotics, except colistin or tigecycline.<sup>21</sup>

## Quantitative Biofilm Formation Assay

Biofilm formation was quantitatively detected using the modified microtiter plate assay as previously described<sup>12</sup> with some modification. Briefly, after overnight incubation of all isolates inoculated into Tryptic-Soy Broth (TSB, Merck, Germany) containing 0.5% glucose at 37°C, cultures were diluted 1:40 in TSB containing 0.5% glucose. Then, 100  $\mu$ L of  $10^8$  CFU/mL of *A. baumannii* and an equal volume of TSB containing 0.5% glucose were added to each well of 96-well polystyrene microtiter plates (Nunc, Denmark), and incubated at 37°C for 48 hours. The well containing 200  $\mu$ L of TSB with 0.5% glucose was considered as a negative control. Wells were slowly washed three times with Phosphate Buffered Saline (PBS; pH 7.2; Invitrogen, USA), fixed by methyl alcohol (Merck, Germany) for 20 minutes, dried at 20–25°C, and then stained by 0.1% safranin (Merck, Germany). Finally, the optical density (OD) of each well was measured at 490 nm. Optical density cut-off (ODc) was defined as 3X standard deviation above mean OD of the negative control. The isolates were divided into four groups according to their OD results including non biofilm producer ( $OD \leq ODc$ ), weak biofilm producer ( $ODc < OD \leq 2 \times ODc$ ), moderate biofilm producer ( $2 \times ODc < OD \leq 4 \times ODc$ ), and strong biofilm producer ( $4 \times ODc < OD$ ).

## PCR Detection of Biofilm-Related Genes

All isolates were subjected to PCR assays to detect *bap*, *ompA* and *bla<sub>PER-1</sub>* genes, as previously reported.<sup>3,8,12</sup> The primers used in this study are listed in Table 1.<sup>3,12</sup> PCR was

carried out in a thermocycler (Cyclogene, Techne, UK). A single reaction mixture contained: 5  $\mu$ L (5 ng) of genomic DNA, 1  $\mu$ L (10 pmol) of each primer (Promega, USA), and 12.5  $\mu$ L of PCR HotStarTaq Master Mix (Promega) containing 0.4 mM of each dNTP, 3 mM MgCl<sub>2</sub>, and 0.08 U/ $\mu$ L Taq DNA polymerase in reaction buffer with a final volume of 25  $\mu$ L. Initial denaturation (94°C for 4 min) was followed by 30 cycles of amplification. Each cycle consisted of 94°C for 45 seconds, various annealing temperature for different genes for 45 seconds (Table 1), and 72°C for 45 seconds. A final extension step (72°C for 5 min) completed the amplification. The amplified products were electrophoresed on 1.5% agarose gel (Sigma, USA) and were visualized by ethidium bromide staining (Sigma, USA).

## Statistical Analysis

Data were coded, validated and analysed using the Statistical Package for the Social Sciences (SPSS), version 22 (SPSS Inc., Chicago, IL, USA). Frequency, percentage, arithmetic mean and median were used to present the data. Numerical data were compared using the Student's *t*-test, and categorical data were compared using the chi-squared test or Fisher's exact test, where appropriate. All comparisons were two-tailed, and *p* values <0.05 were considered statistically significant. Binary logistic regression analysis with adjusted odds ratio (aOR) and antecedent 95% confidence intervals (CI) were used to identify potential risk factors.

## Ethical Considerations

The present study followed the principles of the Helsinki Declaration and its amendments. Ethical approval was obtained from the Institutional Review Board (IRB) of National Liver Institute (NLI) (Protocol No. 201,807,358). All the participants were informed about the purpose of the study and written consent was obtained from all participants.

**Table 1** Sequences of Primers Used in This Study for PCR Assays for Detection of Biofilm-Related Genes in *A. baumannii* Isolates (n = 94 Isolates)

Primers	Nucleotide Sequence (5'– 3')	Annealing Temp. (°C)	Amplicon Size (bp)
<i>bap</i> -F <i>bap</i> -R	TGCTGACAGTGACGTAGAACCACA TGCAACTAGTGGAATAGCAGCCCCA	55	1225
<i>ompA</i> -F <i>ompA</i> -R	CGCTTCTGCTGGTGCTGAAT CGTGACAGTAGCGTTAGGGTA	58	531
<i>bla<sub>PER-1</sub></i> -F <i>bla<sub>PER-1</sub></i> -R	GCAACTGCTGCAATACTCGG ATGTGCGACCACAGTACCAG	55	927

## Results

### Isolates Distribution

All 94 non-duplicate clinical isolates of *A. baumannii* during the study period were isolated from non-redundant patients (38 isolates from females and 56 isolates from males). The age of patients ranged from 41 to 71 years with an average of  $55.5 \pm 12.3$  and a median of 53.6 years. The specimens from which organisms were isolated included respiratory specimens (50%; 42.6% from sputum and 7.4% from endotracheal aspirate samples), blood (33%), and wound swab (17%). The majority of clinical isolates were collected from ICU (67%) followed by medical ward (16%), burn unit (10.6%), and surgical ward (6.4%).

### Characteristics of Biofilm Producers

Among all *A. baumannii* isolates tested for biofilm formation, 66 (70.1%) isolates were biofilm producers and 28 (29.9%) isolates were non-biofilm producers. Of 66 biofilm-producing strains, 16% isolates were considered as weak biofilm producers, 34% as moderate biofilm producers and 20.2% as strong biofilm producers.

The distribution of different biofilm-producing *A. baumannii* phenotypes among clinical specimen types and hospital wards is presented in Figure 1. Interestingly, 66.7% (44 isolates) of biofilm producers and 53.6% (15 isolates) of non-biofilm producers were isolated from ICU. Besides, more than half of biofilm producers (23 isolates; 52%) and non-biofilm producers (16 isolates; 57%) were isolated from blood and sputum specimens, respectively.

All *A. baumannii* were investigated for the presence of biofilm-related genes (*bap*, *ompA* and *bla<sub>PER-1</sub>* genes). The PCR assays yielded positive results for one gene in 41 (43.7%) isolates, and two genes in 18 (19.1%) isolates. *A. baumannii* isolates harbouring *ompA* gene with the rate of 63.8% (60 isolates) were the most prevalent genotype, while the prevalence of *bap* and *bla<sub>PER-1</sub>* genes among isolates was 13.8% (13 isolates) and 10.6% (10 isolates), respectively.

The demographic and clinical characteristics of patients infected by biofilm-forming and non-biofilm-forming *A. baumannii* isolates are presented in Table 2. There were no significant differences in the demographic characteristics, types of specimen, ward admission, comorbidities, or invasive procedures between the two groups. However, the length of hospital stay, the previous antibiotic therapy, and the presence of *ompA* or *bap* genes were

statistically significant risk factors associated with infections by biofilm-producing isolates (p values: 0.04, 0.015, 0.002, 0.012, respectively).

### Biofilm Production and Antibiotic Resistance

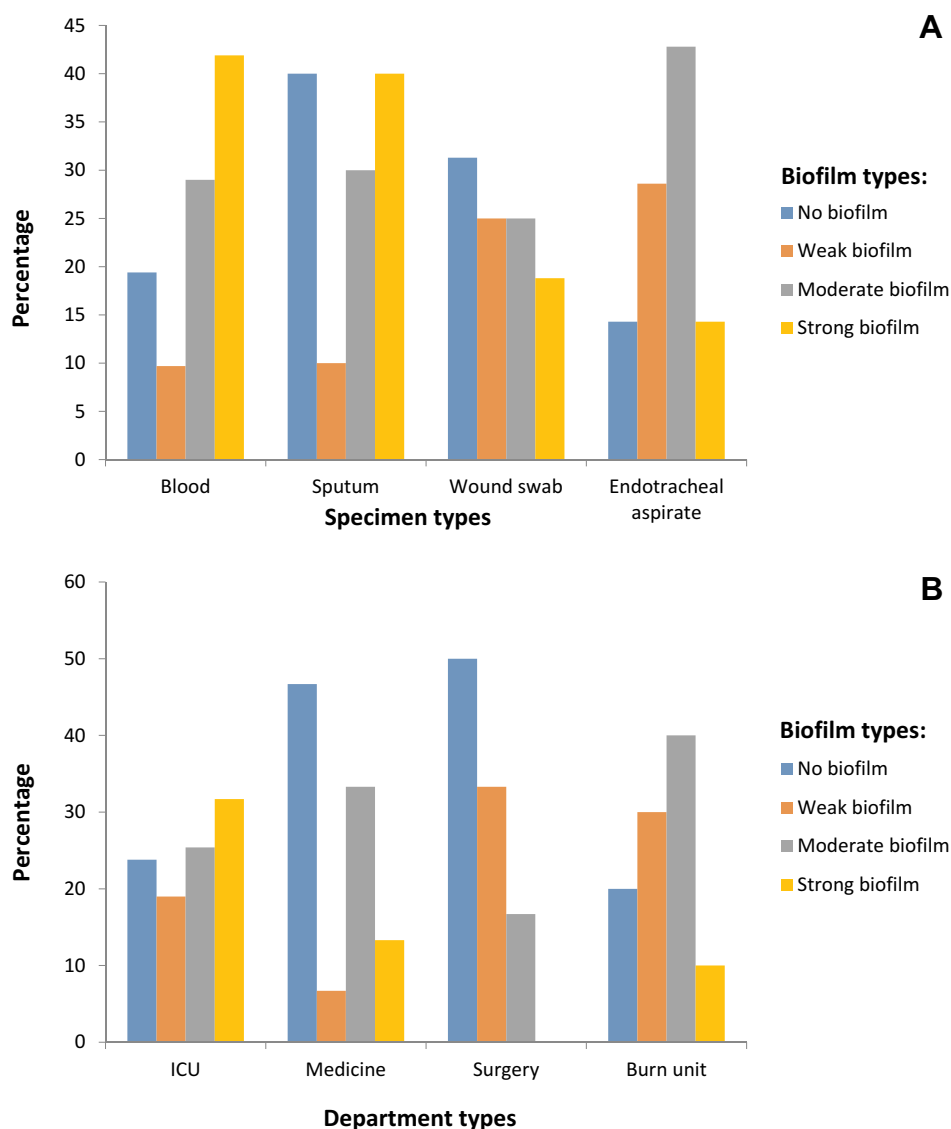
To assess whether biofilm formation is associated with any particular antimicrobial resistance pattern, all isolates of different biofilm-producing phenotypes with various resistance profiles for 16 antibiotics were compared (Table 3). The biofilm-producing isolates showed a statistically significant higher resistance rate to ceftazidime, ampicillin/sulbactam, piperacillin/tazobactam, piperacillin, gentamicin, trimethoprim/sulfamethoxazole, tigecycline and imipenem (p = 0.041, <0.001, 0.006, 0.034, 0.028, 0.002, 0.002, and 0.02, respectively). Interestingly, the presence of MDR (p = 0.017) and XDR (p = 0.002) was significantly associated with biofilm-producing capability of the isolates, compared to non-biofilm producing capabilities.

### Potential Factors Associated with Biofilm-Producing Capability

The multiple logistic regression analysis with adjusted odds ratio (aOR) and antecedent 95% confidence intervals (CI) identified potential risk factors linked to biofilm producing ability among all isolates (Table 4). The length of hospital stay (aOR = 0.023, 95% CI: 0.003–0.043), the presence of *ompA* gene (aOR = 0.286, 95% CI: 0.115–0.456) or *bap* gene (aOR = 0.346, 95% CI: 0.081–0.610), ampicillin/sulbactam resistance (aOR = 1, 95% CI: 0.652–1.348), and the presence of MDR (aOR: –0.329, 95% CI: –0.457 – –0.112) or XDR (aOR = –0.252, 95% CI: –0.328 – –0.047) were considered significant risk factors associated with biofilm producing isolates.

## Discussion

The emerging trend of biofilm formation among MDR and XDR *A. baumannii* clinical isolates and its association with serious nosocomial infections has been considered a global health crisis.<sup>1,6,15</sup> In this study, the observed overall rate of biofilm-producing *A. baumannii* was 70% and half of them were moderate to strong biofilm producers. These results are consistent with previous findings from other studies, which found values ranging from 50% to 76%, worldwide.<sup>2–6</sup> Previous epidemiological studies have showed that biofilm formation is the most important virulence factor facilitating the chronicity and endemicity of



**Figure 1** The distribution of different biofilm-producing *A. baumannii* phenotypes among (A) Clinical specimen's types and (B) Hospital department's types.

*A. baumannii* strains in both nosocomial infections and hospital environment.<sup>3,5,12,14,22,23</sup> It is noteworthy that >70% of biofilm-producing *A. baumannii* isolates in this study were obtained from hospitalized patients in ICU wards. This finding would provide an additional explanation for the role of biofilm-producing *A. baumannii* in nosocomial infections and outbreaks among high-risk ICU patients.<sup>3,5</sup>

Several epidemiological and molecular studies investigated the various *A. baumannii* repertoires of biofilm-related virulence genes and proteins which contribute to the microbial ability to adhere and form biofilms on diverse surfaces and environments.<sup>8–16</sup> Previous reports have

demonstrated that *bap* and *ompA* genes are the most prevalent genotypes in clinical and environmental isolates. In this study, about two thirds of isolates harboured *ompA* gene, while only 14% of isolates were positive for *bap* gene. These results are consistent with previous findings from other studies. Zeighami et al<sup>3</sup> investigated a battery of biofilm-related genes in 100 MDR *A. baumannii* nosocomial isolates from Iranian ICU patients, and found that 81% of isolates were positive for *ompA* gene. Reports from other countries revealed a prevalence rate of 84.4% in Thailand,<sup>12</sup> 76.5% in Korea<sup>13</sup> and 68.8% in Taiwan.<sup>14</sup> These findings highlight the crucial role of *ompA* gene in the attachment of this



**Table 2** Demographic and Clinical Characteristics of Study Patients Infected by Biofilm- and Non-Biofilm-Forming *A. baumannii* Nosocomial Isolates

Characteristics	Biofilm-Forming (n = 66)	Non Biofilm-Forming (n = 28)	p value
Age, median (IQR), years	53.01 53.5–62	54.9 54–63	0.5
Male sex, No (%)	43 (65.2)	13 (46.4)	0.094
Type of specimen, No (%)			
Blood	25 (37.9)	6 (21.4)	0.122
Sputum	24 (36.4)	16 (57.1)	0.065
Pus	11 (16.7)	5 (17.9)	0.881
ETA	6 (9.1)	1 (3.6)	0.356
Ward admission, No (%)			
ICU	48 (72.7)	15 (53.6)	0.073
Medical	8 (12.1)	7 (25)	0.12
Surgical	3 (4.5)	3 (10.7)	0.262
Burn unit	8 (12.1)	2 (7.1)	0.474
Comorbid conditions, No (%)	33 (50)	8 (28.6)	0.057
Invasive procedures, No (%)	34 (51.5)	14 (50)	0.895
Length of hospital stay, median (IQR), years	8.04 (2–14)	6.07 (4–8)	0.044
Prior Antibiotic therapy, No (%)	57 (86.4)	18 (64.3)	0.015
PCR amplification of: No (%)			
omp gene	50 (75.8)	10 (35.7)	0.0002
bap gene	13 (19.7)	0	0.012
bla <sub>PER-1</sub> gene	6 (9.1)	4 (14.3)	0.457
No of genes: No (%)			
No genes	24 (36.4)	11 (39.3)	0.791
One gene	30 (45.5)	11 (39.3)	0.581
2 genes	12 (18.2)	6 (21.4)	0.712

pathogen to human epithelial cells and development of biofilms. Besides, other studies reported the association of *ompA* gene harbouring and antimicrobial resistance to individual antibiotics such as cefotaxime, aztreonam, ciprofloxacin, and imipenem via specific efflux pumps.<sup>12,14,16</sup>

Due to the continuous exposure to significant selective pressure in the hospital environment, *A. baumannii* usually develop acquired resistance to several antibiotic classes and subclasses through a wide variety of mechanisms, including efflux pumps, porin expression, antibiotic target mutations, and drug-inactivating enzymes.<sup>24</sup> In this study, similar to previous reports,  $\geq 70\%$  of isolates were resistant to cephalosporins, aminoglycosides, carbapenems, and fluoroquinolones, which are commonly used in clinical practice. Besides, the prevalence rate of MDR and XDR isolates in this study was 28% and 73%, respectively. Worldwide, the high prevalence rates of MDR clinical isolates have been

reported, ranging from 21% to 95%.<sup>3,12–15,23</sup> Although determining the prevalence of XDR *A. baumannii* is challenging, and not fully addressed, carbapenem resistance has been considered one of the hallmarks of XDR *A. baumannii*.<sup>25</sup> According to CDC/NHSN, the overall rate of carbapenem-resistant isolates is 47–64% in USA.<sup>26</sup> Reports from other parts of the world showed a prevalence rate of 49% in Europe,<sup>27</sup> 40–60% in Southeast Asia<sup>28</sup> and 40–80% in Latin America.<sup>29</sup> The overall resistance rate to tigecycline and colistin in this study is higher than that reported in previous studies.<sup>15,25</sup> This finding is worrisome and of great concern as these two antibiotics have been considered as the last resort drugs for the treatment of *A. baumannii* nosocomial infections in either single or combined chemotherapeutic regimens.<sup>7,25</sup>

It is worth noting that the resistance rate to individual antibiotics as well as the prevalence rate of MDR and

**Table 3** Comparison of Antimicrobial Resistance Patterns of Biofilm-Forming and Non-Biofilm-Forming *A. baumannii* Nosocomial Isolates

Antimicrobial Agent	All Isolates (n = 94) No. (%)	Biofilm-Forming (n = 66) No. (%)	Non Biofilm-Forming (n = 28) No. (%)	p value
Ceftazidime	75 (79.8)	57 (86.4)	18 (64.3)	<b>0.041</b>
Cefotaxime	80 (85.1)	57 (86.4)	23 (82.1)	0.594
Cefepime	71 (75.5)	53 (80.3)	18 (64.3)	0.101
Ampicillin/sulbactam	84 (89.4)	66 (100)	18 (64.3)	<b>&lt;0.001</b>
Piperacillin/tazobactam	69 (73.4)	51 (77.3)	18 (64.3)	<b>0.006</b>
Piperacillin	86 (91.5)	63 (95.5)	23 (82.1)	<b>0.034</b>
Gentamycin	84 (89.4)	62 (93.9)	22 (78.6)	<b>0.028</b>
Amikacin	73 (77.7)	50 (75.8)	23 (82.1)	0.505
Tobramycin	91 (96.8)	63 (95.5)	28 (100)	0.257
Ciprofloxacin	80 (85.1)	59 (89.4)	21 (75)	0.074
Gatifloxacin	84 (89.4)	61 (92.4)	23 (82.1)	0.141
Trimethoprim/ sulfamethoxazole	78 (83)	60 (90.9)	18 (64.3)	<b>0.002</b>
Imipenem	75 (80)	57 (86.4)	18 (64.3)	<b>0.02</b>
Meropenem	65 (69.1)	47 (71.2)	18 (64.3)	0.51
Tigecycline	37 (39.4)	30 (45.5)	7 (25)	<b>0.002</b>
Colistin	35 (37.2)	25 (37.9)	10 (35.7)	0.841
MDR	26 (27.7)	23 (34.8)	3 (10.7)	<b>0.017</b>
XDR	68 (72.3)	54 (81.8)	14 (50)	<b>0.002</b>

**Note:** Bold values indicate the statistically significant values.

XDR in biofilm-producing isolates in this study was higher than that in non-biofilm producers. Previous studies have reported a positive relationship between biofilm formation and antibiotic resistance in *A. baumannii* isolates with higher resistance rates to aminoglycosides, carbapenems, and sulphonamides. For example, Durate et al<sup>25</sup> found that the isolates resistant to gentamicin and tobramycin were more frequently able to form biofilms (74% and 73.3%, respectively). Thummeepak et al<sup>12</sup> found that 125 (81.7%) of 153 gentamicin resistant clinical isolates were biofilm producers. A study performed on clinical isolates from patients with nosocomial infections in three Iranian hospitals found that 92% of 155 biofilm-forming isolates were

MDR.<sup>30</sup> Moreover, Zeighami et al<sup>3</sup> reported that 49 of 100 biofilm-producing *A. baumannii* clinical isolates were strong biofilm producers and XDR.

To better understand the pathogenic and epidemiologic role of biofilm-producing isolates, regression analysis was used to identify the potential risk factors linked to biofilm-producing ability among all isolates. The risk factors associated significantly with biofilm production of nosocomial isolates in this study were the length of hospital stay ( $P = 0.03$ ), the presence of *ompA* gene ( $P = 0.001$ ) or *bap* gene ( $P = 0.01$ ), ampicillin/sulbactam resistance ( $P = <0.001$ ), and the presence of MDR ( $P = 0.01$ ) or XDR ( $P = 0.02$ ). Although part of these findings have already been described in previous studies as risk factors linked to hospital-acquired infection by various nosocomial pathogens,<sup>4,5,26,31</sup> our results could be considered as useful predictors for the acquisition of nosocomial infections by biofilm-producing *A. baumannii* clinical isolates. These findings would help clinicians design necessary actions for prudent antibiotic policies and effective infection control measure inside hospitals.

This study had some limitations. Firstly, environmental isolates were not included and biofilm-related genes other than *ompA*, *bap* and *bla<sub>PER-1</sub>* genes were not investigated.

**Table 4** Multivariate Analysis of Factors Associated with Biofilm-Forming *A. baumannii* Infections

Variable	Adjusted OR (95% CI)	p value
Length of Hospital Stay	0.023 (0.003–0.043)	0.034
<i>ompA</i> gene	0.286 (0.115–0.456)	0.001
<i>bap</i> gene	0.346 (0.081–0.610)	0.011
Ampicillin/sulbactam	1 (0.652–1.348)	<0.001
MDR	–0.329 (–0.457 – –0.112)	0.012
XDR	–0.252 (–0.328 – –0.047)	0.018

However, this study could be considered as the first epidemiological report including the most prevalent biofilm genetic determinants among nosocomial isolates in our region. Secondly, the lack of molecular genotyping and genome sequencing in this study may have limited its ability to deeply explore the pathogenic role of other virulence markers genes. Finally, this was a single centre study. Therefore, our findings may not be generalized to other settings. Further molecular-based epidemiological multi-centre studies of longer surveillance duration are necessary to better understand the phenotypic and genotypic correlations between biofilm-related virulence genes and antimicrobial resistance. These studies are necessary to help determine national priorities for local interventions.

## Conclusion

The high prevalence of biofilm-producing MDR and XDR nosocomial isolates in this study is worrisome and alarming. The high resistance rate to tigecycline and colistin is challenging and of great concern. Our results highlighted the importance of the length of hospital stay, the presence of *ompA* or *bap* genes, ampicillin/sulbactam resistance, and the presence of MDR or XDR as potential risk factors associated with biofilm-producing *A. baumannii* clinical isolates. These findings could be beneficial for health authorities and decision makers to better control the continuous selection and transfer of this serious phenotype inside hospitals and to improve the quality of patients' care.

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## Disclosure

The authors declare no conflicts of interests for this work.

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