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Distribution of virulence genes involved in biofilm formation in multi-drug resistant *Acinetobacter baumannii* clinical isolates

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Summary. *Acinetobacter baumannii* is an opportunistic bacterial pathogen that is the major cause of hospital-acquired infections. It has been shown that *A. baumannii* with high biofilm formation increases the risk of acquiring infection. In this study, the prevalence of virulence genes involved in biofilm formation was determined in 225 *A. baumannii* clinical isolates from three hospitals in Thailand. Most of the isolates were multidrug-resistant *A. baumannii* strains (86.2%). Among all isolates, 76.9% (173/225) showed biofilm formation ability. The association between biofilm forming ability and gentamicin resistance was found ($P < 0.05$). The presence of virulence genes, *epsA*, *bap*, *ompA*, *bfmS* and bla_{PER-1} genes, was investigated by PCR. The prevalence of *ompA*, *bfmS*, *bap*, bla_{PER-1} and *epsA* genes among the isolated strains was 84.4%, 84%, 48%, 30.2%, respectively. Biofilm formation related genes, *ompA* and *bap* were associated with multidrug-resistant *A. baumannii* strains. The result of this study revealed that a high prevalence of biofilm-forming phenotypes among *A. baumannii* strains obtained from different hospitals. Effective strategies to prevent infection due to *A. baumannii* that produce biofilms are therefore needed. [**Int Microbiol** 19(2):121-129 (2016)]

Keywords: *Acinetobacter baumannii* · biofilms · virulence genes

Introduction

Acinetobacter baumannii is a Gram-negative bacterium that causes a variety of diseases. It exists especially in health care settings such as hospital environments. The emergence of multidrug-resistant *A. baumannii* strains is considered as a major and immediate threat to public health worldwide. One of the major factors involved in bacterial resistance to antimicrobials, chronic infections or survival in varying environments is the ability to form biofilms. There are a variety of virulence determinants involved in biofilm formation of *A. baumannii*. This bacterium produces a molecule called the biofilm-associated protein (BAP), which is encoded by the *bap* gene [21]. BAP contributes to the initiation of biofilm production after *A. baumannii* attaches to a particular surface [14,19]. The outer membrane protein (OmpA), encoded by the *ompA* gene, is an adhesion molecule that functions during the attachment to human epithelial cells and induces biofilm formation [10]. *Acinetobacter baumannii* produces a polysaccharide export outer membrane protein, called exopolysac-

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charide or EPS, which is encoded by *epsA*. EPS accumulates on the cell surface and provides protection to the cells against the harsh external environment [27,31]. Production of EPS is involved in the aggregation of bacteria which is associated with biofilm formation in many bacteria [33]. In addition, Lee et al. [16] 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_{PER-1} gene. Recently, a two-component system (BfmS/BfmR) has been identified which is needed for biofilm formation on polystyrene surfaces [32]. The *A. baumannii* 17978 type strain, with an inactivated *bfmS*, showed a reduction in biofilm formation [17]. To date, the mechanisms by which virulence determinants contribute to biofilm formation and antibiotic resistance still remain unclear. The aim of this study was to determine the association of biofilm formation, antibiotic resistance phenotype and virulence genes in *A. baumannii* clinical isolates.

Materials and methods

Bacterial isolation and identification of *Acinetobacter baumannii*. A total of 225 individual clinical isolates was collected from 3 tertiary hospitals in 3 different provinces in Thailand over the 12 month period from November 2013 to October 2014. All *A. baumannii* isolates were collected from multiple collection sites, including sputum, urine, pus, blood, pleural fluid, ascetic fluid and wound. All isolates were identified as *A. baumannii* by using biochemical tests, detecting of 16S rRNA gene [22] and the intrinsic bla_{OXA-51} gene [1,5]. *Acinetobacter baumannii* ATCC 19606 was used as a control.

Determination of antimicrobial susceptibilities. The antibiotic susceptibility was analyzed by the disc diffusion method (Oxoid). The concentrations of the antibiotics in the discs (expressed in µg) were: amikacin (30), cefotaxime (30), ceftazidime (30), ceftriaxone (30), cefepime (30), ciprofloxacin (5), gentamicin (10), imipenem (10), meropenem (10), trimethoprim/sulfamethoxazole (1.25/23.75), tetracycline (30), cefoperazone/sulbactam (105), and piperacillin/tazobactam (100/10). The Petri dishes were incubated at 35 °C for 24 h. The zones of inhibition determined whether the microorganism was susceptible, intermediately resistant, or resistant to each antibiotic. The results were interpreted according to the CLSI [6].

Detection of biofilm formation. Quantitative microtiter plate assays for biofilm formation were performed as described by Brossard and Campagnari [4] with some modification. One hundred µl of 10^8 CFU/ml of *A. baumannii* and an equal volum of 2× Luria Bertain (LB) broth supplemented with 20% glucose were added to each well in 96-well polystyrene microtiter plates (Nunc, Denmark). The plates were incubated overnight at 37 °C. After incubation, the cultures were gently removed. The wells were washed three times with phosphate buffered saline. The adherent cells were fixed with absolute methanol for 10 min and stained with 0.4 % crystal violet for 15 min, and washed three times with sterile distilled water and then air-dried. Afterward, the plates were filled with 250 µl of 33 % acetic acid for 15 min. The absorbance at OD_{595} nm was determined. All experiments were performed in three independent assays each repeated in triplicate. The mean optical density at 595 nm (OD_{595}) of the non-biofilm producer *E. coli* DH5α was used as the OD cut-off value (OD_c). The OD results of all tested strains were divided into the following four groups: (I) $OD \leq OD_c$ = non biofilm Producer; (II) $OD_c < OD \leq 2\times OD_c$ = weak biofilm Producer; (III) $2\times OD_c < OD \leq 4 \times OD_c$ = moderate biofilm Producer; and (IV) $4\times OD_c < OD$ = strong biofilm producer [35].

Table 1. List of primers for detection of virulence genes used in this study

Target gene	Primer sequence	Tm (°C)	References
epsA	AGCAAGTGGTTATCCAATCG ACCAGACTCACCCATTACAT	50	[31]
ompA	CGCTTCTGCTGGTGCTGAAT CGTGCAGTAGCGTTAGGGTA	50	[31]
bla_{PER-1}	ATGAATGTCATTATAAAAGC AATTTGGGCTTAGGGCAAGAAA	50	[16]
bap	TACTTCCAATCCAATGCTAGGGAGGGTACCAATGCAG TTATCCACTTCCAATGATCAGCAACCAAACCGCTAC	65	[12]
bfmS	TTGCTCGAACTTCCAATTTATTATAC TTATGCAGGTGCTTTTTTATTGGTC	53	[17]
16S rDNA	AGAGTTTGATCCTGGCTCAG ACGGCTACCTTGTTACGACTT	58	[22]
bla_{OXA-51}	TAATGCTTTGATCGGCCTTG TGGATTGCACTTCATCTTGG	52	[5]

Detection of virulence genes by PCR. The presence of *epsA*, *bap*, *ompA*, *bfmS* and bla_{PER-1} genes was detected with primers as shown in Table 1. DNA was extracted from all the isolates by boiling. Each PCR was performed in triplicate in a thermocycler with a PCR condition as described previously [12,16,17,31]. PCR products were analyzed by electrophoresis in 1% agarose gel containing 0.5 µg/ml ethidium bromide.

Statistical analyses. All statistical analyses were performed using Stata (Stata12.0 Corporation, USA). Non-parametric Kruskal–Wallis test and Dunn's test were performed to compare the median value among multiple groups. The difference of biofilm biomass between two groups was compared by using Mann–Whitney U test. Fisher's exact test was used to access differences between frequencies. *P*-values < 0.05 were considered to be statistically significant.

Results

Biofilm formation by clinical *Acinetobacter baumannii* isolates. All *A. baumannii* isolates were tested for the ability to form biofilms. The mean OD_{595} value for the negative control *Escherichia coli* DH5α was 0.221 ± 0.072 and this value was used as the optical density cut-off value (ODc). The classification of biofilm based on ODc revealed that $OD_{595} \leq 0.221$ = non-biofilm; $0.221 < OD_{595} \leq 0.442$ = weak biofilm; $0.442 < OD_{595} \leq 0.884$ = moderate biofilm; $OD_{595} > 0.884$ = strong biofilm. Among all isolates, 23.1% were non-biofilm producers, while the majority were biofilm producers (76.9%). The number of weak biofilm producers was 46 (20.4%), with 74 moderate biofilm producers (32.9 %) and 53 strong biofilm producers (23.6%). The median OD_{595} and interquartile range (IQR) value for non-biofilm formers was 0.148 (0.091, 0.187), for weak biofilm formers, 0.332 (0.297, 0.404), moderate biofilm formers, 0.620 (0.494, 0.720) and strong biofilm formers, 1.170 (0.991, 1.430). We found that 173 of 225 isolates (76.9 %) were more capable of forming biofilms than the DH5α strain with a median biofilm biomass of 0.624 (0.432, 0.949).

Distributions of biofilm-formers in various sources and wards. All 225 *A. baumannii* isolates were obtained from sputum (81.8 %), pus (7.5 %), urine (4.9 %) and other specimens (obtained from skin, blood, coccyx, catheter or pleural fluid) (5.8%). The proportion of strong biofilm producers of the other specimens was 46.2%, of urine, 45.5%,

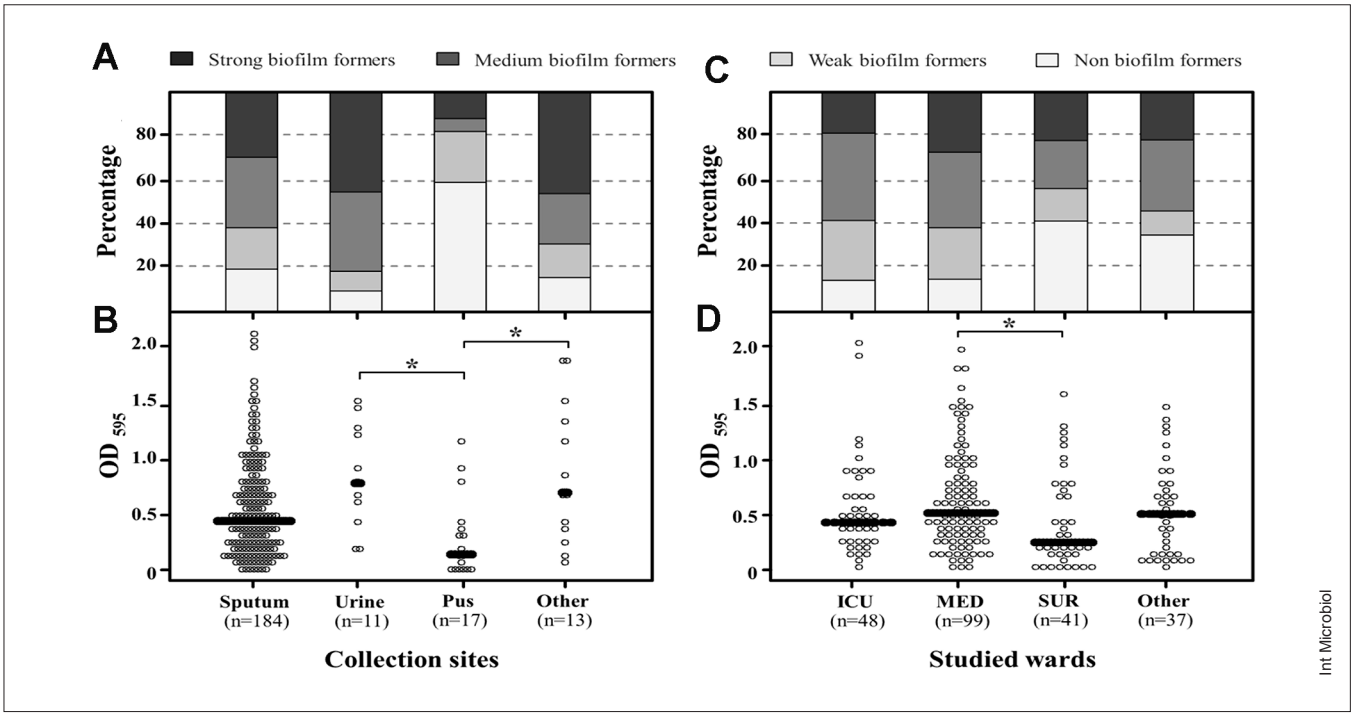

Fig. 1. Characterization of biofilm production in 225 clinical isolates of *Acinetobacter baumannii*. (**A** and **C**) Relative composition of each biofilm formation level from different sources and wards. Each individual bar represents the proportion that contains different biofilm status. (**B** and **D**) The median of biofilm biomass (OD_{595}) in clinical isolates from different sources and wards. Each data point is representative of the mean OD_{595} of independent triplicates of each individual isolates. The line bar (black) represents the median of OD_{595}. ICU, Intensive Care Unit; MED, Medicine; SUR, Surgical; and Other: monk ward, coronary care unit, trauma ward, pediatric ward and outpatient department. Asterisks (*) indicate differences that are statistically significant; Kruskal–Wallis test followed by Dunn's multiple comparison post-test; $P < 0.05$.

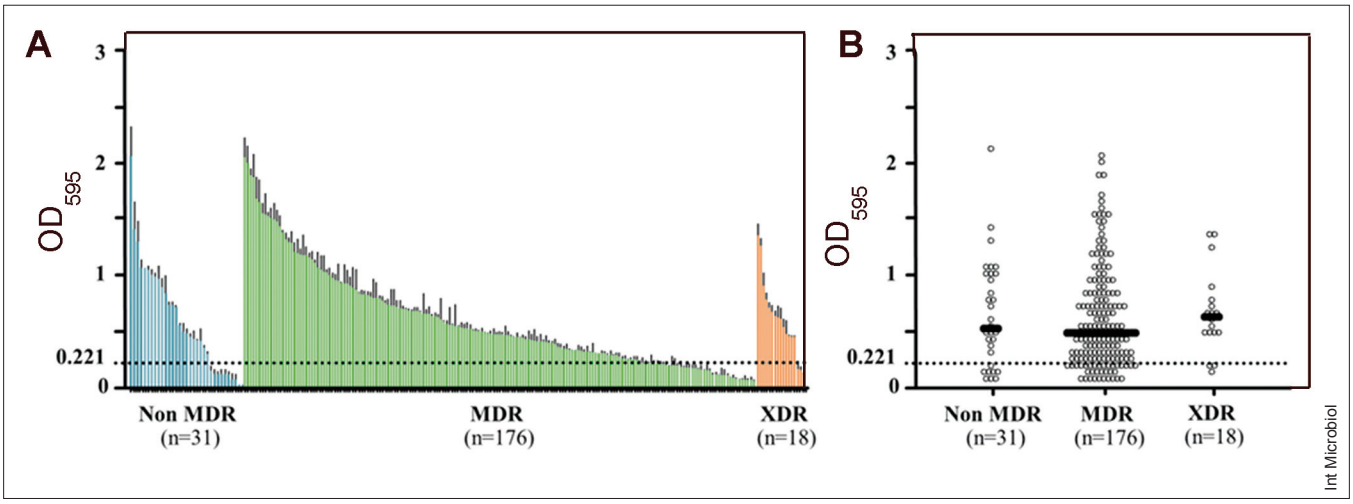

Fig. 2. Biofilm produced (OD595) on polystyrene by 225 isolates of *Acinetobacter baumannii* with different drug susceptibility patterns. (**A**) Biofilm formation of non MDR, MDR and XDR strains are represented by blue, green and orang bars, respectively. Data shown are the means of a triplicate plus standard deviation. (**B**) The open circle dot represents the mean of independent triplicate of each isolate. The line bar (black) indicates the median OD_{595} of each group with differences antibiotic resistance pattern. The dashed lines correspond to the cut-off value (ODc).

sputum, 32.6%, and pus, 11.8% (Fig. 1A). The comparison of biofilm biomass (OD_{595}) among the various sources of specimens by the Kruskal–Wallis test showed significant differences among the groups ($P < 0.05$). The median (IQR) of isolates obtained from urine [0.799 (0.489, 1.300)] and other specimens [0.720 (0.416, 1.330)] were higher than pus [0.186 (0.065, 0.423)] with *P*-values less than 0.05 (Dunn's test) (Fig. 1B).

The prevalence of strong biofilm producers was similar among the strains recovered from different wards, ranging from 18.8% to 27.3% (Fig. 1C). However, analysis of the biofilm forming capacity of the isolates obtained from various wards revealed a statistically significant difference in OD_{595} between medical and surgical wards ($P < 0.05$; Kruskal–Wallis and Dunn's tests) (Fig. 1D).

Association of biofilm-forming capability with antibiotic resistance phenotype. All isolates were tested for their antibiotic susceptibility toward 13 antibiotics. The majority of isolates were resistant to ciprofloxacin (84.4%). The *A. baumannii* isolated strains were also resistant to amikacin (54.2%), cefotaxime (76.9%), ceftazidime (82.2%), ceftriaxone (81.3%), cefepime (67.6%), gentamicin (68%), imipenem (79.6%), meropenem (78.7%), trimethoprim/sulfamethoxazole (54.2%), tetracycline (62.7%) cefoperazone/sulbactam (20.4%) and piperacillin/tazobactam (79.1%). All isolates were defined as being multidrug resistant *A. baumannii* (MDRAB) when there was resistance to more than three antibiotic classes. The incidence of MDRAB was 86.2 % (194/225). Among 194 of MDRAB isolates, 150 (77.3 %) were biofilm-forming strains while 74.2 % (23/31) of non-MDRAB also produced biofilms ($P = 0.654$; Fisher's exact test). This finding was supported by analysis of the median OD_{595} among three drug resistance patterns (Fig. 2). As illustrated in Fig. 2B, the median (IQR) of non-MDRAB, MDRAB and XDRAB were 0.500 (0.157, 0.990), 0.461 (0.245, 0.794) and 0.605 (0.444, 0.770), respectively. There was no significant difference among the groups ($P = 0.536$; Kruskal–Wallis test).

The association between biofilm forming ability and individual drug resistance of *A. baumannii* was evaluated. The resistance rates of most antibiotics were found to be similar in both biofilm-forming and non-biofilm forming groups with a *P*-value ranging from 0.191 to 1.000. Of the 153 gentamicin resistant isolates, 125 (81.7%) strains were biofilm producers while only 48 of 72 (66.7%) of gentamicin susceptible strains were biofilm producers ($P = 0.017$; Fisher's exact test). This result was also confirmed by using Mann–Whitney U-test to compare the median (IQR) of OD_{595} between drug resistant and susceptible groups. In A. baumannii that resistance to gentamicin had a significantly higher ability to build biofilms when compared with gentamicin sensitive groups ($P < 0.05$) (Table 2). In contrast, the tetracycline susceptible isolates tended to form greater biofilm biomass than resistant strains ($P < 0.001$; Mann–Whitney U-test) (Table 2). However, the incidence rate of biofilm former in tetracycline susceptible

strains was similar to that in resistant strains (82.1 vs. 73.8%) ($P = 0.191$; Fisher's exact test). For other antibiotics, no statistical correlation was observed (Table 2).

Relationship of biofilm production and the presence of different virulence genes. Polymerase chain reaction (PCR) was utilized to investigate the presence of the *epsA*, *bap*, *ompA*, *bfmS* and bla_{PER-1} genes in all *A. baumannii* isolates. The electrophoresis analysis showed that the amplicon sizes of *epsA*, *bap*, *ompA*, *bfmS* and bla_{PER-1} genes were 451, 531, 927, 1225 and 1428 bp, respectively (data not shown). The most common virulence genes identified were *ompA* (84.4%) and *bfmS* (84%). The prevalence of genes *bap*, bla_{PER-1} and *epsA* genes among the isolated strains was 48%, 30.2% and 22.2%, respectively. Among the 225 *A. baumannii* isolates, all 5 virulence genes were present in 9 isolates (4%). The association between biofilm formed on the microtiter plate and the presence of virulence genes was also tested, using Mann–Whitney U test. The presence or absence of *epsA*, *bfmS* and bla_{PER-1} was not associated with the biofilm biomass ($P > 0.05$; Fig. 3C–E). The strains lacking bap or ompA genes form stronger biofilms than isolates carrying bap or ompA ($P < 0.05$; Fig. 3A,B). However, the frequency of epsA, bfmS, blaPER-1, bap and ompA was no significant difference between biofilm producers and non-biofilm producers with a P-value more than 0.05 (0.253, 0.281, 0.393, 0.117 and 0.199, respectively, Fisher's exact test). We also examined the differences among various virulence gene patterns in their ability to produce biofilm. The number of virulence genes contributed to the trend of decreased biofilm biomass, but this was not statistically significant ($P > 0.05$; Kruskal–Wallis test) (Fig. 3F).

Correlation between virulence genes and antibiotic resistance patterns. The association between the presence of virulence genes and MDR status was evaluated. There is no statistical relationship between MDRAB and any of *A. baumannii* harbored *epsA*, *bfmS* and bla_{PER-1} genes (Table 3). The genes encoding BAP were present at a higher frequency in MDRAB than in non-MDRAB strains ($P < 0.05$) (Table 3). Gene *ompA* was present in 169 of 194 (87.1%) MDRAB isolates versus only 21 of 31 (67.74%) of non-MDRAB strains ($P < 0.05$). The correlation between the presence of *ompA* and resistance to thirteen antimicrobials was also evaluated. The strains carrying *ompA* were found at a higher prevalence of resistance at least one drug from five antimicronial categories including aminoglycosides, cephems, fluoroquinolones, carbapenems and penicillins + β-lactamase inhibitors than the strains without this gene ($P < 0.05$; (Table 4). The number of virulence genes present in the isolates was a statistically significant predictor of multiple drug resistance phenotype (risk ratio, 1.16; 95% CI, 1.11 to 2.42; $P = 0.011$).

Discussion

Biofilm production in clinical *Acinetobacter baumannii*. The ability of bacteria to form biofilm is regarded as an important virulence factor which plays a significant role in the bacteria's persistence and antibiotic resistance [20]. In our work, we determined the biofilm formation, antibiotic susceptibility patterns and virulence genes among 225 clinical isolates. We found that more than seventy percent of studied *A. baumannii* showed biofilm formation ability. Our findings agree closely with those previously reported in [8] which showed that 75% of clinical *A. baumannii* isolates were positive for biofilm production, although different criteria were used to interpret biofilm status.

Correlation of biofilm among specimen types and wards. Clinical isolates recovered from urine and other sources (skin tissue, blood, coccyx, catheter and pleural fluids) were shown to have a significantly higher ability to form biofilms compared to those recovered from a pus source. This result agrees with a previous study which reported that *A. baumannii* obtained from urine specimens produced biofilms with a greater biomass [8]. Urinary tract pathogens may have abilities to adhere and form biofilms in flowing environments, resulting in persistent infections. Our analysis also found significant differences in biofilm producing capacity among isolates from various hospital wards. The ability to form a biofilm on an abiotic of clinical isolates provides biofilm associated infection due to the attachment and colonization on medical device surfaces, such as urinary catheters [7].

Biofilm and antibiotic resistance. *Acinetobacter baumannii* is a major global health problem. In the past decade, high prevalence rates of MDRAB clinical isolates have been reported worldwide, ranging from 21–95% [15,18,34]. Similarly to other reports, we observed a high prevalence rate of MDRAB in this study. Previous studies reported that the MDR phenotype of pathogens as well as *A. baumannii* was linked to biofilm producing ability [13,28]. In contrast, our results indicate that the MDR and XDR phenotype has no association with biofilm producing ability. The ability of bacte-

Table 2. Correlation between biofilm biomass (OD_{595}) and drug resistance phenotype in all *Acinetobacter baumannii* isolates

Antibiotics susceptibility	Median (IQR) of OD_{595}		*P*-value[a]
	Susceptible	Resistant	
amikacin	0.440 (0.252, 0.790)	0.496 (0.246, 0.890)	0.208
cefotaxime	0.481 (0.209, 0.921)	0.470 (0.277, 0.790)	0.942
ceftazidime	0.494 (0.211, 0.980)	0.470 (0.248, 0.790)	0.651
ceftriaxone	0.531 (0.252, 0.970)	0.465 (0.246, 0.790)	0.386
cefepime	0.515 (0.210, 0.830)	0.463 (0.258, 0.803)	0.893
ciprofloxacin	0.489 (0.157, 0.970)	0.471 (0.252, 0.790)	0.926
gentamicin	0.400 (0.154, 0.769)	0.515 (0.297, 0.860)	0.012*
imipenem	0.541 (0.279, 0.900)	0.461 (0.241, 0.790)	0.386
meropenem	0.510 (0.278, 0.860)	0.465 (0.241, 0.799)	0.648
TMX/SXT	0.521 (0.297, 0.901)	0.445 (0.237, 0.720)	0.166
tetracycline	0.692 (0.348, 1.055)	0.444 (0.213, 0.669)	<0.001*
cefoperazone/sulbactam	0.461 (0.244, 0.814)	0.553 (0.268, 0.890)	0.303
piperacilin/tazobactam	0.462 (0.252, 0.790)	0.473 (0.246, 0.820)	0.928

[a]*P*-values represent the comparison of median OD_{595} of bacterial strains between two groups (Mann–Whitney U-test). An asterisk (*) indicates the significance (*P*-value < 0.05).

ria to form biofilm may be associated with antibiotic resistance at the level of the individual. For example, Naparstek et al. [23] studied the biofilm production in *Klebsiella pneumoniae* and they concluded that high-level gentamicin resistant strains show greater biofilm biomass compared with populations which have low-level resistance (median value of 0.15 versus 0.07, respectively) [23]. In 2016, Duarte et al. observed that *A. baumannii* isolates resistant to gentamicin and tobramycin were more frequently able to form biofilms than susceptible strains [8]. In our study we found that strains positive for biofilm formation were more frequently resistant to gentamicin.

In addition, the biofilm biomass of gentamicin resistance isolates was greater than susceptible groups (Table 2). Similar

Table 3. Relationship between virulence genes and antibiotic susceptibility patterns in all tested *Acinetobacter baumannii* isolates

The present of virulence genes	All *A. baumannii* isolates n = 225 (%)		*P*-value[a]
	Non MDRAB (n = 31)	MDRAB (n = 194)	
bap	8 (25.81)	100 (51.55)	0.011*
ompA	21 (67.74)	169 (87.11)	0.013*
epsA	7 (22.58)	43 (22.16)	1.000
bfmS	26 (83.87)	163 (84.02)	1.000
bla_{PER-1}	8 (25.81)	60 (30.93)	0.676

[a]*P*-values represent the comparison between non MDRAB and MDRAB groups (Fisher's exact test; $P < 0.05$). An asterisk (*) indicates the statistical significance (*P*-value < 0.05).

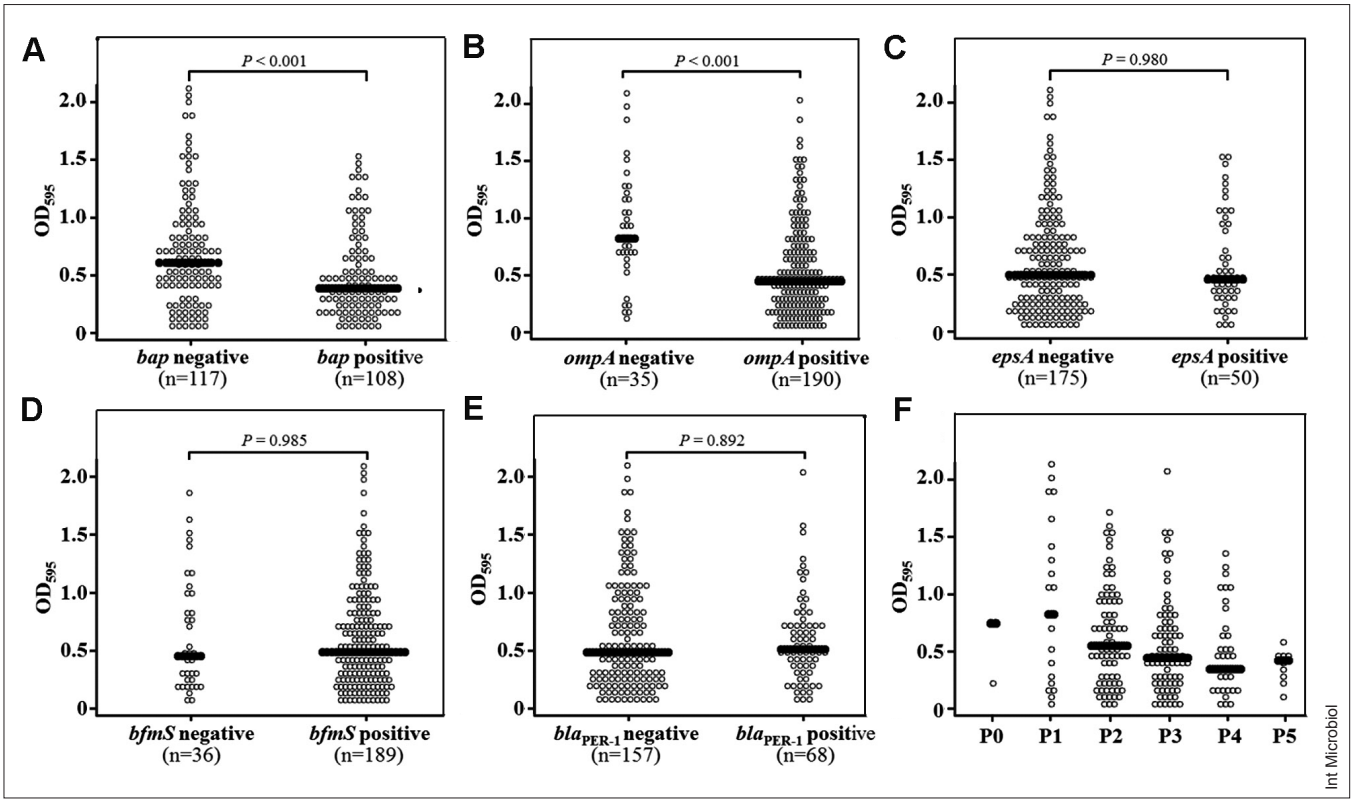

Fig. 3. The correlation between in vitro biofilm formation and virulence genes. (**A–E**) Biofilm forming capacity (OD_{595}) of bacteria harboring and not harboring individual virulence genes. Asterisks (*) indicate differences that are statistically significant; Mann–Whitney U-test. (**F**) OD_{595} among various virulence gene patterns P0; the absent of gene and P1–P5; the present of one to five genes, respectively. Each data point is the mean OD_{595} of independent triplicates of each individual isolates. The line bar (black) represents the median OD595 of each group.

results have also been reported for *Pseudomonas aeruginosa*, where strains characterized as gentamicin resistant showed a significant increase in biofilm production when compared to susceptible strains [30]. Our analysis of biofilm forming ability with gentamicin resistance phenotypes has provided positive statistical association findings.

We proposed that this phenotypic correlation may be due to biofilm-associated and gentamicin resistance determinants are co-located on the same plasmid or genomic island. However, we found only an association between negative biofilm forming ability and tetracycline resistance phenotype. This finding differs from previous study in which a negative correlation between biofilm forming ability and antibiotic resistance to each of 20 antibiotics was reported [24]. The mechanism of this association was not clear but the expression of bla_{TEM-1} was reported to block biofilm formation via the bacterial adhesion interfering [11].

Biofilm and virulence genes. We found that *A. baumannii* isolates harboring virulence genes did not promote biofilm forming ability on polystyrene, while the presence of *bap* or *ompA* showed an inverse correlation. Although many reports have demonstrated that biofilm associated genes, including *bap*, *ompA*, *epsA*, *bfmS* and bla_{PER-1}, were responsible for the biofilm development of only certain selected *A. baumannii* strains [10,14,17,19,21,27,31,32], these reports did not fully characterize their functions in a diverse range of other strains and on different surfaces. Moreover, bla_{PER-1} and *ompA* were not over-expressed in biofilm cells as previously analyzed indicating that these genes are not fully required for biofilm production in some strains [25,26]. This suggests that other key factors or strain-dependent variations contribute to biofilm forming phenotypes in diverse biotic or abiotic surfaces. [3,9].

Virulence gene and MDR phenotype. Although, the OmpA of *A. baumannii* was found to be essential for the development of biofilms and attachment to human epithelial cells [10]. Its involvement in antimicrobial resistance phenotype was also reported [29]. In agreement with our results, the

Table 4. The correlation between the presence of *ompA* and antibiotic resistance phenotype in all tested *Acinetobacter baumannii* isolates

Antibiotics resistance	All *A. baumannii* isolates n = 225 (%)		*P*-value [a]
	ompA⁻ (n=35)	*ompA*⁺ (n=190)	
AMINOGLYCOSIDES			
amikacin	17 (48.57)	105 (55.26)	0.468
gentamicin	18 (51.43)	135 (71.05)	0.030*
CEPHEMS			
cefotaxime	22 (62.56)	151 (79.47)	0.048*
ceftazidime	23 (65.71)	162 (85.26)	0.014*
ceftriaxone	22 (62.57)	161 (84.74)	0.004*
cefepime	21 (60.00)	131 (68.95)	0.328
FLUOROQUINOLONES			
ciprofloxacin	25 (71.43)	165 (86.84)	0.038*
carbapenems			
imipenem	20 (57.14)	159 (83.68)	0.001*
meropenem	21 (60.00)	156 (82.11)	0.006*
FOLATE PATHWAY INHIBITORS			
TMX/SXT	19 (54.29)	103 (54.21)	1.000
PENICILLINS + B-LACTAMASE INHIBITORS			
cefoperazone/sulbactam	2 (5.71)	44 (23.16)	0.021*
piperacilin/tazobactam	23 (65.71)	155 (81.58)	0.042*

[a] *P*-values were analyzed using Fisher's exact test. Asterisks (*) assign statistical significance results ($P < 0.05$) between the *ompA*-negative and the *ompA*-positive groups.

association between MDR phenotype and the presence of *bap* and *ompA* genes was found. It is possible that due to OmpA being a β-barrel porin, antibiotics may be transferred from the periplasm through the outer membrane and then couples with inner membrane efflux pumps. We also found that *A. baumannii* carrying *ompA* were associated with individual drug resistant phenotype (e.g., cefotaxime, ciprofloxacin, and imipenem). This finding is consistent with a previous report which indicated that, in *A. baumannii* ATCC 17978, OmpA was involved in resistance to chloramphenicol, aztreonam and nalidixic acid [29].

We conclude that in this study we found a high prevalence of MDRAB and no difference in its ability to form biofilms when compared with non-MDRAB. Our data indicate that non-MDRAB strains have the ability to form biofilms, and biofilm formation might help these strains adapt or persist during infections. The presence of tested virulence genes does not seem to be related to biofilm formation of *A. baumannii* on a plastic surface. Interestingly, two of those genes, especially *ompA*, was associated with antibiotic resistant phenotypes. The transcriptional or translational analysis of virulence genes can provide good data to confirm their association with biofilm or antibiotic resistance phenotypes which must be further analyzed.

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Competing interests. None declared.

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