

ORIGINAL ARTICLE

Virulence Profile of *Acinetobacter baumannii* Isolated from Ventilator-Associated Pneumonia Cases

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ABSTRACT

Key words:

Adherence, Biofilm, Serum resistance, Virulence, *Acinetobacter baumannii*

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Background: *Acinetobacter baumannii* (*A. baumannii*) is an opportunistic bacterium with high antimicrobial resistance and a complex set of virulence factors. It can cause hospital-acquired infections such as ventilator-associated-pneumonia (VAP). The study aimed to detect some virulence factors for *A. baumannii* isolated from VAP samples phenotypically and genotypically and relating them to antimicrobial resistance. **Methodology:** Thirty *A. baumannii* VAP isolates were obtained and tested for antimicrobial sensitivity using the disc-diffusion method. Polystyrene adherence replica method was used to detect bacterial adherence. Capsules were screened using the Anthony capsular staining method. Biofilm forming capacity and serum resistance were also determined. Fifteen genes encoding for either adhesive or non-adhesive virulence factors were detected using multiplex PCR. **Results:** All *A. baumannii* isolates showed a high antimicrobial resistance pattern with 60% being multi-drug resistant. Most isolates showed polystyrene adherence; 33.3%, 20% and 26.7% were of high, moderate and low adherence, respectively. All isolates were biofilm formers; 73.3% were moderate or strong biofilm formers. All isolates except 4 were encapsulated and 86.7% showed serum resistance. Most isolates had a high number of virulence genes which correlated significantly with the detected phenotypic virulence factors and multi-drug resistance. **Conclusion:** *A. baumannii* VAP isolates could- in a variable degree - adhere to polystyrene, produce biofilm, produce capsule and have serum resistance. Most of these virulence factors were associated with multidrug-resistance and mostly correlated with adhesive and/or non-adhesive virulence genes. A virulence profile was introduced for these isolates that highlights the hazard of this highly resistant and virulent bacterium.

INTRODUCTION

In Intensive Care Units (ICUs), mechanical ventilators are frequently used to prolong patient survival. However, patients undergoing mechanical ventilation are susceptible to an array of pulmonary problems such as ventilator-associated pneumonia (VAP). VAP is defined as pneumonia that develops 48 to 72 hours following endotracheal intubation and affects 9–27% of individuals who have been intubated. This infection not only has impacts on the health outcomes of patients but also raises healthcare costs for both patients and the healthcare system ¹.

One of the main causes of mortality in ICUs is nosocomial infections, and particularly VAP caused by multidrug-resistant (MDR) bacteria, such as *Acinetobacter baumannii* (*A. baumannii*) which is characterized by rapid resistance development to the majority of known antimicrobials leading to extensive drug resistance, and even pan drug resistance².

Moreover, *A. baumannii* is known for a high virulent potential as it encompasses a complex set of virulent factors. The capsule is a predominant virulence factor providing an anti-phagocytic effect and causes evasion of the host immune system³. Bacterial surface hydrophobicity is crucial for attachment to polymers and adherence to various abiotic surfaces including plastics, catheters and prosthetic devices⁴. Biofilm formation is another pathogenic aspect that facilitates colonization on prostheses, accompanied by high antibiotic resistance. Besides that, *A. baumannii* has the ability of serum resistance where it can resist the complement inactivation in normal human serum⁵. Other virulence factors include: siderophores, outer membrane proteins and phospholipases⁶.

There are multiple genes regulating these virulence factors which may encode for adhesive or non-adhesive types. Adhesive virulence factors comprise of: curli fibers (*csg*), fibronectin receptor (*fbn*) and polysaccharide coatings as group II and III capsules (*kpsMT*) and different

fimbriae encoding genes; S and F1C fimbriae (*sfa/focDE*), fimbriae Dr-antigen family (*afa/dra*), P fimbriae (*pap* genes; *papC* and *papGI-III*), type 1 fimbriae (*fimH*). Non-adhesive factors include serum resistance (*traT*), colicin V production (*cvaC*), cytotoxic necrotizing factors (*cnf1* and *cnf2*), in addition to the siderophores/iron acquisition systems as yersiniabactin (*fyuA*) and aerobactin (*iutA*). Moreover, pathogenicity-associated islands (*PAI*) which are virulence factors gene blocks that present a coordinated mechanism for horizontal virulence genes transfer between strains^{7,8}.

The current study aimed to detect the presence of some virulence factors for *A. baumannii* isolated from VAP clinical samples both phenotypically and genotypically and to relate these virulence factors to antimicrobial resistance. This provides an outline for the virulence profile of these isolates.

METHODOLOGY

Bacterial Isolates:

Samples were collected from hospitalized and mechanically ventilated cases for more than 48 hours. Patients were eligible for the study if pneumonia was suspected based on clinical criteria. A total of 110 samples were collected from cases admitted to ICUs at different hospitals in Alexandria, Egypt. VAP was defined as infection of lower respiratory tract after 2 days of mechanical ventilation⁹. The study included *A. baumannii* isolates only. Conventional biochemical tests were used for bacterial identification¹⁰ and confirmed with PCR amplification of 16S-23S ribosomal RNA gene¹¹. We stored the isolates at -80 °C for upcoming investigations.

Antibiotic susceptibility testing:

Antibacterial susceptibility for all the isolates was tested using the disc diffusion method. The used antibiotics discs were purchased from Oxoid, London, UK. The discs included were, Ampicillin/sulbactam (10µg/10µg), Piperacillin/tazobactam (100 µg/10 µg), Ceftriaxone (30 µg), Cefepime (30 µg), Ceftazidime (30 µg), Imipenem (10µg), Meropenem (10µg), Gentamycin (10µg), Tobramycin (10µg), Amikacin (30µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Gatifloxacin (30 µg), and Cotrimoxazole (30 µg). Disc diffusion methodology and the results interpretation were done as stated by the Clinical and Laboratory Standards Institute (CLSI) recommendations 2022¹².

Phenotypic detection of some virulence factors:

Adherence to polystyrene Replica method illustrating bacterial hydrophobicity.

This phenotypic test was done according to the method stated by Uber et al.¹³ with some modifications. Briefly, the bacteria were cultured and incubated for 48hrs on nutrient agar plates. The plates were photographed. A sterile plate of polystyrene was firmly

pressed onto the cultured agar plate for a few seconds. The pressed polystyrene plate is considered a replica-plate that was washed for 2 min using distilled water and allowed to air drying. The plate was stained using 1% crystal violet for 1 min so the adherent cells to the polystyrene surface were stained. Comparison between the photographs of the bacterial growth on the nutrient agar and the stained replicas on polystyrene plates took place in order to score the polystyrene adherence pattern. *Escherichia coli* strain ATCC 25922 provided the negative control. Classification of the isolates was performed visually into high, moderate, low and no adherence.

Serum resistance:

Serum resistance was determined according to King et al.⁵. Overnight culture for the isolates was grown in LB broth. The culture of each isolate was centrifuged where the cells were separated and washed. The cells were resuspended in phosphate buffer saline PBS and the turbidity was adjusted to 10⁷ CFU/ml. The bacterial suspension was added to an equal volume of normal human serum (NHS) then incubated at 37 °C. A serial dilution was constructed; every 30 min an inoculum was taken from the incubated suspension on LB agar plate and then diluted 2-fold. Incubation of LB agar plates was done for 24 h at 37 °C. The colonies count of each plate was stated as CFU/ml. The serum bactericidal effect was calculated through the percentage increase of bacterial count after 3 h and denoted as percentage survival (% survival). The isolates were considered serum resistant when % survival was 50% or higher, and considered serum sensitive, with % survival was less than 20% after incubation of 3 h.

Anthony capsule staining for Capsule Examination:

The capsule was detected for each isolate using Anthony capsular staining method¹⁴. Briefly, each isolate was cultured in nutrient broth till early exponential phase when optical density (OD₆₀₀) is equivalent to 0.3-0.4. On a glass slide, 20µl of each the bacterial suspension and skimmed milk were blended. The smear was dried for 2 min then stained with 1% crystal violet. Using copper sulfate solution (20%), the smear was washed. The capsule was detected visually using a light microscope as a transparent zone between a purple background and purple stained cells.

Biofilm forming capacity:

To measure the isolates biofilm forming capacity, quantitative assay was performed as stated by Stepanovic et al.¹⁵ with minor changes. In TSB, the isolates were cultured overnight, then cultures turbidity was set at 10⁶ CFU/ml. For each isolate 200µl of the bacterial suspension were transported to microtiter plate wells then incubated for 48hrs at 37°C. Negative control was a microtiter plate well contains only broth with no isolates. The broth was discarded then the wells were washed by PBS (pH 7.2) and dried. Biofilm fixation was done for 20 min using 99% methanol and then

washed with PBS. Crystal violet solution (1%) was used for biofilm staining (15 min, 200µl). The dye was discarded, washed then the stain of biofilm was eluted using glacial acetic acid (33%). The eluted stain optical density was measured using BioTek ELISA plate reader at 570 nm. This assay was performed three times for each condition. The average optical density of the negative control was calculated and denoted OD_c, whereas the average optical density for each isolate was also calculated and denoted as OD. The biofilm forming capacity was interpreted qualitatively into strong, moderate, weak or none-biofilm producers as stated by Hassan et al. ¹⁶.

Genotypic detection of virulence related genes by the Polymerase Chain Reaction (PCR):

The boiling method was used for bacterial DNA extraction ¹⁷. The selected adhesive and non-adhesive

virulence genes of *A. baumannii* were detected using multiplex PCR in four sets using Veriti Thermal Cycler (Applied Biosystems). Primer sequences utilized and the amplicon sizes are shown in (Table 1) ^{4,18}.

PCR runs included a negative control where DNA extract was not included, and water was added instead. The thermal profile of amplification was: 95 °C for 4 minutes for initial denaturation then 50 seconds denaturation at 95°C, 60 seconds annealing (sets A, B & C were at 58°C while set D was at 56°C) and 45 seconds extension at 72°C (30 cycles). Finally, a cycle of final extension at 72°C for 4 minutes was performed. Agarose gel electrophoresis (1.5%) was used to separate and visualize the amplicons.

Table 1: Primers sequences of the detected virulence related genes.

PCR set	Gene	Primer	Primer Sequence (5'-3')	PCR product (bp)
Set A	<i>afa/draBC</i>	afa1	GCTGGGCAGCAAACCTGATAACTCTC	750
		afa2	CATCAAGCTGTTTGTTCGTCCGCCG	
	<i>cnf1</i>	cnf1	AAGATGGAGTTTCCTATGCAGGAG	498
		cnf2	CATTCAGAGTCCTGCCCTCATTATT	
	<i>csgA</i>	M464	ACTCTGACTTGACTATTACC	200
	M465	AGATGCAGTCTGGTCAAC		
Set B	<i>cvaC</i>	CoIV-CF	CACACACAAACGGGAGCTGTT	680
		CoIV-CR	CTTCCCGCAGCATAGTTCCAT	
	<i>fyuA</i>	FyuA F	TGATTAACCCCGCGACGGGAA	880
		FyuA R	CGCAGTAGGCACGATGTTGTA	
	<i>iutA</i>	AerJ F	GGCTGGACATCATGGGAACCTGG	300
AerJ R		CGTCGGGAACGGGTAGAATCG		
Set C	<i>knf2</i>	knf2a	AATCTAATTAAGAGAAC	543
		knf2b	CATGCTTTGTATATCTA	
	<i>kpsMT II</i>	kpsII F	GCGCATTTGCTGATACTGTTG	272
		kpsII R	CATCCAGACGATAAGCATGAGCA	
	<i>PAI</i>	RPAi F	GGACATCCTGTTACAGCGCGCA	930
RPAi R		TCGCCACCAATCACAGCCGAAC		
Set D	<i>papC</i>	pap1	GACGGCTGTACTGCAGGGTGTGGCG	328
		pap2	ATATCCTTTCTGCAGGGATGCAATA	
	<i>fimH</i>	FimH F	TGCAGAACGGATAAGCCGTGG	508
		FimH R	GCAGTCACCTGCCCTCCGGTA	
	<i>ibeA</i>	ibe10 F	AGGCAGGTGTGCGCCGCGTAC	170
ibe10 R		TGGTGCTCCGGCAAACCATGC		
<i>papG II, III</i>	pGf	CTGTAATTACGGAAGTGATTTCTG	1070	
	pGr	ACTATCCGGCTCCGGATAAACCAT		
	sfa1	CTCCGGAGAACTGGGTGCATCTTAC	410	
<i>sfa/focDE</i>	sfa2	CGGAGGAGTAATTACAAACCTGGCA		
	traT	TraT F	GGTGTGGTGCATGAGCACAG	290
		TraT R	CACGGTTTCAGCCATCCCTGAG	

Statistical analysis:

All statistical tests were conducted in RStudio by R programming platform (version R.4.2.3). Ggplot2, corrplot, finalfit and Rstatix are the main used packages. Normality of data was determined statistically by *Shapiro-Wilk* test and visually by QQ plots. Categorical

variables are presented in count and percent. Fisher Exact or Chi-square tests were used as appropriate. Spearman's correlation was performed between different parameters. For all tests, statistical significance was considered when p-value ≤ 0.05.

RESULTS

Bacterial Isolates

The total number of the collected bacterial isolates was 110, over a period of time of 6 months, from patients diagnosed with VAP and admitted to ICUs at different hospitals in Alexandria, Egypt. Out of them, 30 *A. baumannii* isolates were included in the current study after confirmed identification.

Antimicrobial susceptibility testing

All 30 *A. baumannii* isolates were tested for their susceptibility against 14 antimicrobials. The highest resistance level was observed against Ciprofloxacin (21/30, 70 %) followed by Imipenem and Meropenem (18/30, 60%), and then Ampicillin/sulbactam (16/30, 53.3%). Resistance to the tested cephalosporines ranged between 36.7-50% while resistance to tested aminoglycosides ranged between 20-50%. Additionally, 30% of isolates were resistant to Levofloxacin, and 23.3% were resistant to Gatifloxacin and Cotrimoxazole while only 20% of isolates showed resistance to Piperacillin/tazobactam. According to definition of MDR¹⁹, 60% (18/30) of *A. baumannii* isolates were categorized MDR.

Phenotypic detection of virulence factors:

Adherence to Polystyrene

Most of the isolates were adherent to polystyrene (10/30 were high, 6/30 were moderate, 8/30 were low),

while only 20% (6/30) were non-adherent. Despite, most of MDR isolates (12/18, 66.7%) showed moderate or high adherence and most of non-MDR isolates (8/12, 66.7%) exhibited no or low adherence, no significant association was observed (Table 2 and Figure 1)

Biofilm formation

All the isolates were biofilm formers of which the majority (22/30, 73.3%) were either moderate or strong biofilm formers. Almost all MDR isolates (16/18, 88.9%) were significantly associated (p= 0.034) with moderate or strong biofilm compared to non-MDR isolates (6/12, 50%) (Table 2).

Capsule production

A total of 26 isolates (86.7%) were encapsulated while the other 4 (13.3%) were non-capsulated. There was not any significant difference in capsule production between MDR and non-MDR isolates (Table 2 and Figure 2)

Serum resistance

Twenty six out of the 30 isolates (86.7%) could highly resist inactivation by complement in NHS. A significant association (p= 0.018) between serum resistance and antibiotic resistance profiles was found. All MDR isolates (18/18, 100%) showed serum resistance while 66.7% of non-MDR isolates were serum resistant (Table 2).

Table 2: Association between antibiotic resistance profiles and different phenotypic characteristics among *A. baumannii* isolates (N= 30).

Phenotypic characteristics		MDR isolates, n= 18	Non-MDR isolates, n= 12	Total, N= 30	^{FE} p-value
Polystyrene Adherence	No or low	6 (33.3)	8 (66.7)	14 (46.7)	0.135
	Moderate or high	12 (66.7)	4 (33.3)	16 (53.3)	
Biofilm	Weak	2 (11.1)	6 (50.0)	8 (26.7)	0.034
	Moderate or strong	16 (88.9)	6 (50.0)	22 (73.3)	
Capsule	Positive	16 (88.9)	10 (83.3)	26 (86.7)	1.000
	Negative	2 (11.1)	2 (16.7)	4 (13.3)	
Serum resistance	Resistant	18 (100.0)	8 (66.7)	26 (86.7)	0.018
	Susceptible	0 (0.0)	4 (33.3)	4 (13.3)	

MDR: multidrug resistant; Data is presented in count and percent.; ^{FE}p: Fisher Exact test
Bold p-value represents statistically significant difference between MDR and non-MDR isolates.

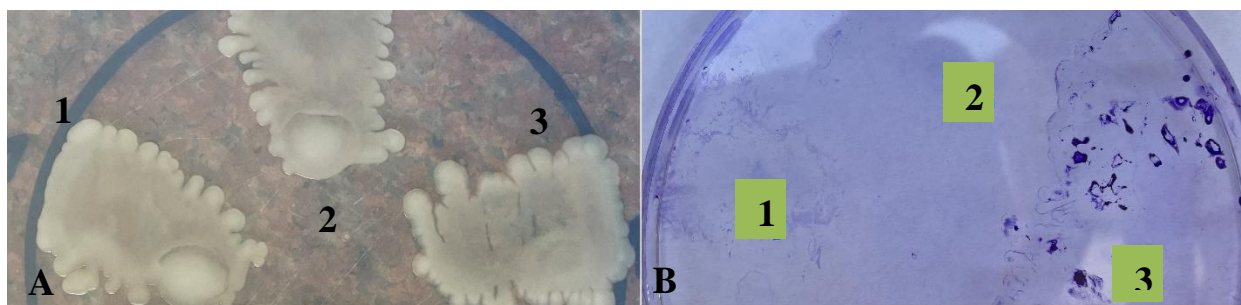


Fig. 1: Adherence to polystyrene. (A) Nutrient agar plate containing 3 *A.baumannii* isolates; (B) polystyrene replica stained with violet crystal. 1- An isolate showing low adherence. 2- *Escherichia coli* ATCC 25922 – negative control – No adherence. 3- An isolate showing high adherence.

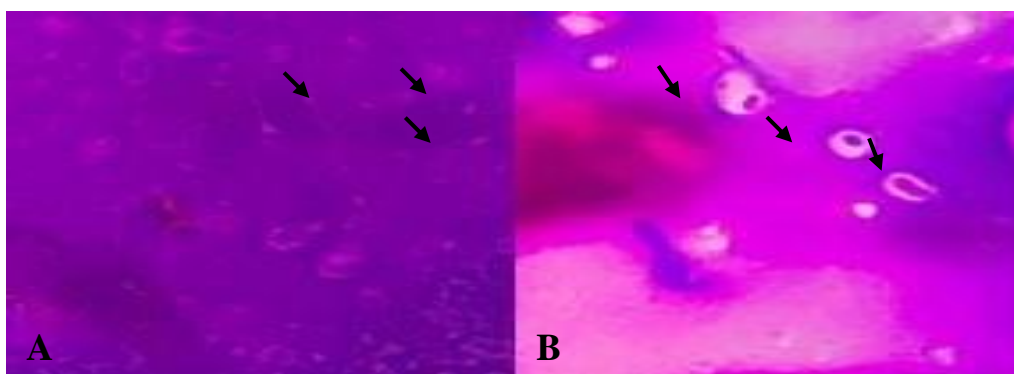


Fig. 2: Light microscope fields by Anthony capsule staining method. (A) Arrows showing a non-capsulated isolate, (B) Arrows showing an encapsulated isolate

Genotypic detection of virulence genes

A total of 15 virulence genes were screened among all 30 *A. baumannii* isolates. Out of them, 7 genes (*afa/draBC*, *fimH*, *papG*, *papC*, *sfa/focDE*, *csgA*, and *kpsMT*) were for adhesive virulence factors while the other 8 genes (*cnf1*, *cnf2*, *cvaC*, *iutA*, *fyuA*, *PAI*, *ibeA*, and *traT*) were for non-adhesive virulence factors. The

iutA gene was detected in 100% of isolates. **Table 3** shows comparison of 15 virulence genes between MDR and non-MDR isolates from which only 3 genes showed significant association ($p \leq 0.05$) with MDR isolates compared to non-MDR. Those 3 genes were *traT*, *afa/draBC* and *csgA* which were observed in 100%, 83.3%, and 44.4% among the MDR isolates.

Table 3: Association between antibiotic resistance profiles and multiple virulence genes among *A. baumannii* isolates (N= 30).

Virulence gene		MDR isolates, n= 18	Non-MDR isolates, n= 12	Total, N= 30	^{FE} p-value
<i>iutA</i>	positive	18 (100)	12 (100)	30 (100)	-
<i>afa/draBC</i>	positive	15 (83.3)	5 (41.7)	20 (66.7)	0.045^a
	negative	3 (16.7)	7 (58.3)	10 (33.3)	
<i>papG</i>	positive	18 (100.0)	9 (75.0)	27 (90.0)	0.054 ^a
	negative	0 (0.0)	3 (25.0)	3 (10.0)	
<i>papC</i>	positive	18 (100.0)	10 (83.3)	28 (93.3)	0.152 ^a
	negative	0 (0.0)	2 (16.7)	2 (6.7)	
<i>sfa/focDE</i>	positive	15 (83.3)	7 (58.3)	22 (73.3)	0.21 ^a
	negative	3 (16.7)	5 (41.7)	8 (26.7)	
<i>csgA</i>	positive	8 (44.4)	1 (8.3)	9 (30.0)	0.049^a
	negative	10 (55.6)	11 (91.7)	21 (70.0)	
<i>fimH</i>	positive	11 (61.1)	5 (41.7)	16 (53.3)	0.501 ^b
	negative	7 (38.9)	7 (58.3)	14 (46.7)	
<i>kpsMT</i>	positive	13 (72.2)	7 (58.3)	20 (66.7)	0.461 ^a
	negative	5 (27.8)	5 (41.7)	10 (33.3)	
<i>cnf1</i>	positive	13 (72.2)	9 (75.0)	22 (73.3)	1.000 ^a
	negative	5 (27.8)	3 (25.0)	8 (26.7)	
<i>cnf2</i>	positive	14 (77.8)	8 (66.7)	22 (73.3)	0.678 ^a
	negative	4 (22.2)	4 (33.3)	8 (26.7)	
<i>cvaC</i>	positive	1 (5.6)	1 (8.3)	2 (6.7)	1.000 ^a
	negative	17 (94.4)	11 (91.7)	28 (93.3)	
<i>fyuA</i>	positive	3 (16.7)	2 (16.7)	5 (16.7)	1.000 ^a
	negative	15 (83.3)	10 (83.3)	25 (83.3)	
<i>PAI</i>	positive	4 (22.2)	5 (41.7)	9 (30.0)	0.418 ^a
	negative	14 (77.8)	7 (58.3)	21 (70.0)	
<i>ibeA</i>	positive	1 (5.6)	1 (8.3)	2 (6.7)	1.000 ^a
	negative	17 (94.4)	11 (91.7)	28 (93.3)	
<i>traT</i>	positive	18 (100.0)	8 (66.7)	26 (86.7)	0.018^a
	negative	0 (0.0)	4 (33.3)	4 (13.3)	

MDR: multidrug resistant; Data is presented in count and percent.; a: Fisher Exact test.; b: Chi-square test. Bold p-value represents statistically significant difference between MDR and non-MDR isolates.

Correlation analyses:

Correlation between different phenotypic traits such as adherence, biofilm, capsule, serum resistance and multiple virulence genes was investigated for significance (**Figure 3**). It was found that polystyrene adherence is positively correlated with 5 virulence genes, *afa/draBC* (rho= 0.78, p <0.001), *sfa/focDE* (rho= 0.74, p< 0.001), *csgA* (rho= 0.71, p= 0.001), *fimH* (rho= 0.78, p< 0.001), and *kpsMT* (rho= 0.78, p <0.001). Strong or moderate biofilm producers were positively correlated with only one gene, *traT* (rho= 0.65, p= 0.016). Furthermore, capsule was not significantly correlated with any of tested virulence genes. On the other hand, serum resistance was positively correlated

with several genes including *papG* (rho= 0.85, p<0.001), *papC* (rho= 0.68, p= 0.005), *sfa/focDE* (rho= 0.65, p= 0.016), and *traT* (rho= 1, p<0.001).

Additionally, between the virulence genes, several significant correlations were observed (**Figure 3**). *afa/draBC* gene was significantly correlated with *sfa/focDE* (rho= 0.85, p< 0.001), *fimH* (rho= 0.76, p<0.001), and *kpsMT* (rho= 0.70, p= 0.002). *papG* gene was significantly associated with *papC* (rho= 0.80, p< 0.001) and *traT* (rho= 0.85, p< 0.001). Moreover, *sfa/focDE* gene was significantly correlated with *fimH* (rho= 0.64, p=0.018), *kpsMT* (rho= 0.85, p< 0.001) and *traT* (rho= 0.65, p= 0.016).

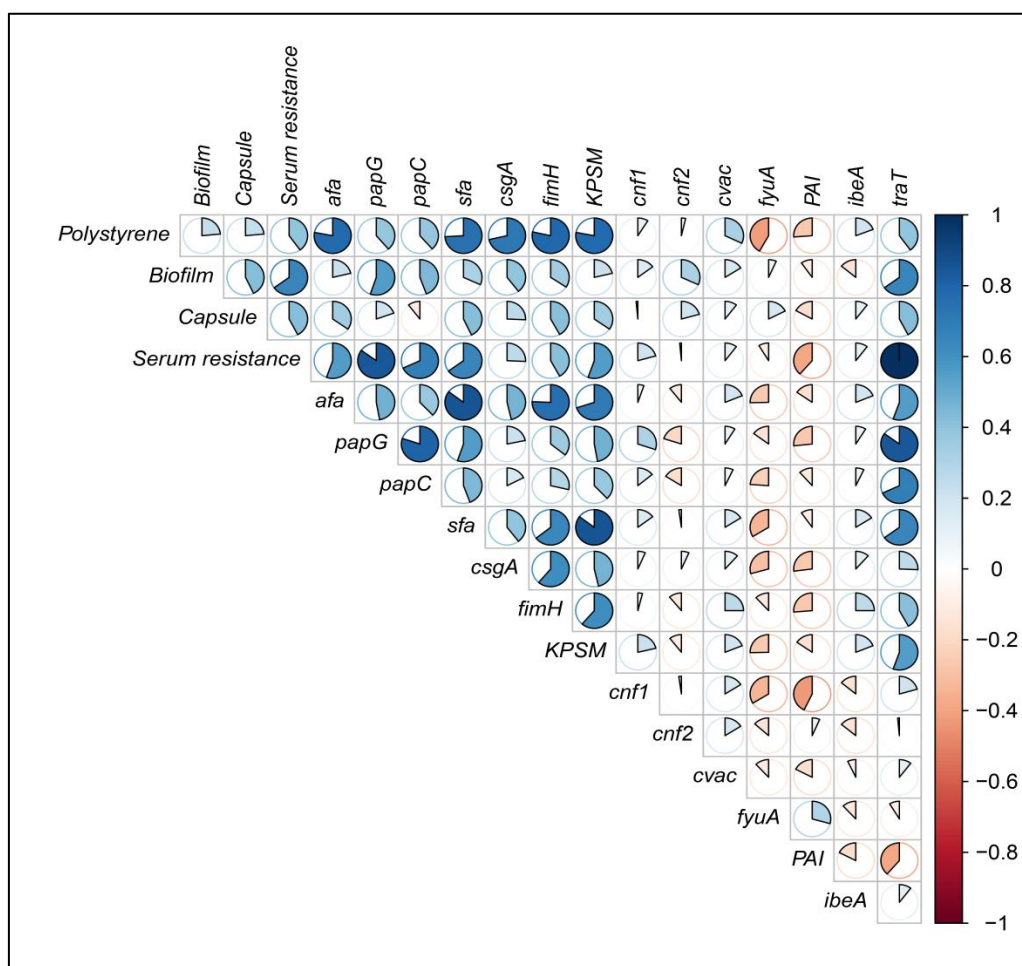


Fig. 3: Correlation matrix between phenotypic traits and virulence genes among the *A. baumannii* isolates (N= 30).

The side bar represents the correlation coefficient which ranges between -1 to +1. A correlation coefficient of 0 indicates that there is no correlation while a correlation coefficient of +1 indicates a perfect positive correlation, and a correlation coefficient of -1 indicates

a perfect negative correlation. **Blue colors** indicate positive correlation, while **red colors** indicate negative correlation. The **intensity of color and the size of the pie** reflect the value of correlation coefficients.

A significant positive correlation was observed ($\rho = 0.63$, $p = 0.0002$) between biofilm mean optical density (OD) and % survival indicating serum resistance (**Figure 4, A**). Another significant positive correlation ($\rho = 0.48$, $p = 0.0068$) between total number of

virulence genes (virulence score) and % survival that indicates serum resistance (**Figure 4, B**). Moreover, there was a very strong positive correlation ($\rho = 0.91$, $p < 0.001$) between number of adhesive genes and polystyrene adherence (**Figure 4, C**)

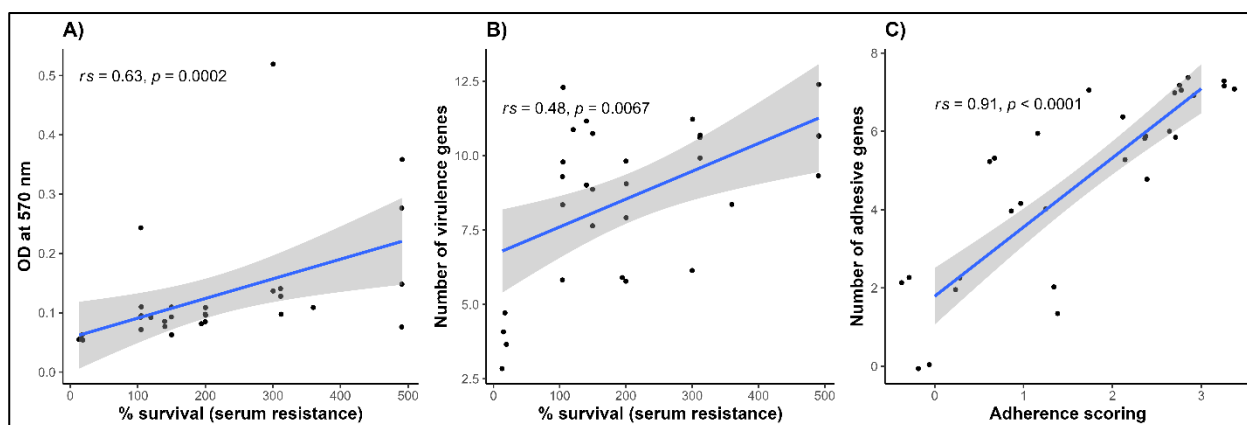


Fig. 4: Scatter plots showing Spearman's correlation (A) between biofilm optical density and % survival (serum resistance), (B) between number of virulence genes and % survival (serum resistance), and (C) between number of adhesive genes and polystyrene adherence scoring.

Polystyrene adherence scoring:

0= non-adherent. 1= low adherent. 2= moderate adherent. 3= high adherent.

rs: Spearman's correlation coefficient, OD: optical density of biofilm

DISCUSSION

A. baumannii is an opportunistic bacterium with high antimicrobial resistance and a complex set of virulence factors. It is also well known for causing hospital acquired infections where it can persist on different surfaces, so it can successfully colonize hospitalized patients e.g. VAP patients²⁰. The current study aimed to detect presence of some virulence factors for *A. baumannii* isolated from VAP clinical samples phenotypically and genotypically and also relate these factors to antimicrobial resistance. This provides an outline for the virulence profile of these isolates.

In this study, out of the 110 VAP isolates 30 (27.3%) were identified as *A. baumannii*. Similar findings were reported by Chi et. al.²¹, where 30% of 109 VAP bacterial isolates were *A. baumannii*. In a 3 year Vietnamien study out of 1500 VAP isolates, 904 (60.2%) were *A. baumannii*²². On the other hand, a lower percentage (15%) for *Acinetobacter* spp. was reported among VAP isolates by Jakribettu et al.²³.

All 30 isolates showed high antimicrobial resistance patterns against the tested antibiotics. Biedenbach et al. observed even higher antimicrobial resistance among their *A. baumannii* VAP isolates where the resistant isolates for cefepime, ceftazidime, imipenem, meronem and piperacillin/tazobactam were 92.9%, 93.1%, 92.3%, 92.5% and 92.7%, respectively. High resistance to

ciprofloxacin (95%) and levofloxacin (90%) was also reported. The least resistance was for aminoglycosides; amikacin 81%, tobramycin was 86.7% and gentamycin 88.5%²².

One of the major concerns of VAP management is the increasing prevalence of MDR pathogens¹⁹. This mainly leads to use of empirical treatment of critically ill patients with a combination of broad spectrum antibiotics, which may lead to higher mortality rates and maintain the increasing resistance cycle^{24, 25}. This study included 60% MDR *A. baumannii* VAP isolates. Kanaan and Khashan revealed high MDR percentage among *A. baumannii* isolated from ICU (75%)²⁶. Bahador et al.²⁷ reported that the MDR *A. baumannii* isolates obtained from 3 different hospitals were 91%, 89%, and 75%.

This work phenotypically detected some virulence factors of *A. baumannii* isolates. The bacterial hydrophobicity of these isolates was detected through polystyrene adherence replica method. Most of the isolates (80%) were adherent to polystyrene among which 33.3%, 20% and 26.7% were of high, moderate, and low adherence, respectively. Uber et al.¹³ declared that 52.6% of the *A. baumannii* isolates were polystyrene adherent; 10.5% were highly adherent, 10.5% were moderately adherent and 20% were of low adherence. Braun and Vidotto⁴ stated that only 53.8 % of their *A. baumannii* strains were adherent to polystyrene.

All the isolates included in this study were biofilm producers; 73.3% were strong or moderate producers whereas 26.7% were weak producers. Almost all the MDR isolates (88.9%) were significantly associated with moderate or strong biofilm. Yang et al.²⁸ found that the vast majority of the studied *A. baumannii* isolates were biofilm producers as follows: 32.5%, 45.4% and 15.6% were strong, moderate, and weak producers, respectively. Their results revealed a statistically significant correlation between biofilm formation and antibiotic resistance. Although Badave and Kulkarni²⁹ reported lower percentage for biofilm formation among their isolates (62.5%), but like our findings they proved a correlation between MDR and biofilm forming isolates. Also, Li et al.³⁰ reported that 99% of their *A. baumannii* could produce biofilm among which 51.5% formed strong biofilm. Qi et al.³¹ stated that 91% of their *A. baumannii* isolates produced biofilm. Unlike our results both reported that antibiotic susceptible, non-MDR isolates are correlated to strong biofilm production.

In our study 86.7% of the *A. baumannii* isolates showed thick capsule and only 13.3% (4 isolates) were non-capsulated. Although 88.9% of MDR isolates were encapsulated, no significant correlation was associated between capsule formation and MDR isolates. Using a different detection methodology, Kon et al.³ showed that 59.6% of their *A. baumannii* isolates were encapsulated while 40.3% were either thin or non-capsulated. Hu et al. declared that 90% of the *A. baumannii* strains produced thick capsules whilst, 10% had thin capsules. All the thickly encapsulated strains were MDR³². This may be explained as the capsular genes expression were positively regulated when subjected to antibiotic stress³³.

Concerning the serum resistance of the isolates, 86.7% (26/30) were serum resistant. Additionally, there was significant correlation between resistance profile and serum resistance, where all the MDR isolates were serum resistant. Similar findings were reported by Guo et al.³⁴, 77.9% of the isolates were serum resistant, besides antibiotic resistant isolates showed stronger serum resistance. Uber et al.¹³ also stated that all the tested isolates were serum resistant, and these isolates were also MDR. King et al.⁵ found that only 42.9% of their *A. baumannii* strains showed serum resistance.

A total of 15 virulence genes were screened among all 30 *A. baumannii* isolates, that included genes for adhesive and non-adhesive virulence factors. The genes *traT*, *afa/draBC* and *csgA* were significantly associated with MDR isolates. The correlation between *traT* gene and MDR isolate is consistent with the phenotypic results of this study.

There was a statistically significant correlation between the fimbriae genes. On one hand, a correlation between *afa/draBC* gene and *sfa/focDE* ($p < 0.001$) and *fimH* ($p < 0.001$). On the other hand, a correlation between

papG gene and *papC*. Also, a statistically significant correlation was found between *afa/draBC* gene and *kpsMT*. These correlations seem to be relevant as they are between fimbriae genes and/or capsular polysaccharide gene^{7,8}.

There was a high number of positive adhesive genes (6-7) that corresponded to high polystyrene adherence. Polystyrene adherence was positively correlated with 3 fimbriae adhesive genes; *afa/draBC* gene, *sfa/focDE* gene and *fimH*, a non-fimbriae adhesive gene; *csgA* and capsular polysaccharide adhesive gene *kpsMT*. It was referred that the presence of fimbriae in *A. baumannii* is critical factor in adherence to plastic surfaces⁴.

Interestingly, there was a strong association between biofilm formation and serum resistance. This was observed both genotypically (strong or moderate biofilm producers with *traT* gene, $p = 0.016$) and phenotypically (biofilm mean optical density (OD) and % survival indicating serum resistance, $p = 0.0002$). This association was previously stated by King et al. and they proposed an explanation; that serum resistance may trigger the bacterial biofilm formation as it potentiates the survival against the host immune defenses. Alternatively, the biofilm matrix may induce serum resistance⁵.

We observed that a high number of positive virulence genes coincided with high serum resistance. Serum resistance was significantly associated with *traT* gene presence (gene encoding for serum resistance). Moreover, it was positively correlated with some fimbriae genes including *papG* ($p < 0.001$), *papC* ($p = 0.005$) and *sfa/focDE* ($p = 0.016$). Uber et al.¹³ also highlighted correlation between serum resistance and different adherence capability of the bacterium suggesting increased capability of adhering to different living and non-living surfaces associated with increasing risk of systemic infections.

CONCLUSION

This study showed that different *A. baumannii* VAP isolates could- in a variable degree -adhere to polystyrene and produce biofilm, produce capsule, and have serum resistance. Moreover, the presence of these virulent factors was associated with MDR. This was also proved with different correlations with adhesive and/or non-adhesive virulence genes. These characteristics introduce a virulence profile for these isolates and provide an alarm as the bacterium is highly capable of contaminating the different hospital surfaces and prosthesis together with colonizing healthcare professionals and patients. This increases the risk of nosocomial opportunistic infections of high drug resistance and host immune system resistance which minimize the treatment facilities. This urgently recommends developing new antibacterial agents and

treatment strategies to combat these highly virulent bacteria.

Declarations

Conflict of interest:

The authors report no conflicts of interest

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All authors contributed equally to this work.

Ethics statement:

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the Medical Research Institute ethical review committee approval has been received with the following approval number E/C. S/N. T5/2019.

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