



Different Virulence Capabilities and *ompA* Expressions in ST2 and ST513 of Multidrug-Resistant *Acinetobacter baumannii*

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Abstract

Successful clones of *Acinetobacter baumannii* cause a variety of nosocomial infections through serum resistance, biofilm formation, and antimicrobial resistance as virulence capabilities. Fifty clinical isolates of multidrug-resistant (MDR) *A. baumannii* were analyzed for clonal relatedness, serum resistance, biofilm formation, and in vivo assays. Furthermore, some virulence genes, sequence variation of *ompA*, and its expression were studied. The MLST (multilocus sequence typing) results showed that there were three sequence types among MDR isolates including ST2 (64%, 32/50), ST513 (30%, 15/50), and ST1 (6%, 3/50). The data showed that the clinical isolates recovered from sputum had mostly high biofilm-formation capacity, while isolates recovered from host interior fluids had high serum resistance. The results of PCR assays and in silico analysis represented patterns of virulence genes and even *ompA* sequence variations among MDR isolates which were clonally dependent. While quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis showed that bacteremia-producing strains in C57/BL6 mice significantly overexpress *ompA* ($P < 0.05$) and have a direct relation with the level of IL-6 in bloodstream of mice. Moreover, the expressions of *ompA* among indistinguishable clones (ST2 or ST513) were clonally independent.

Introduction

Acinetobacter baumannii has emerged as a challenge to the modern therapeutic system because of increasing infections and global spread of multidrug-resistant (MDR) strains [1]. In spite of its clinical importance, the mechanism of

pathogenicity in *A. baumannii* remains uncertain [2]. One of the major mechanisms in the pathogenesis of *A. baumannii* is biofilm formation [3]. This bacterial strategy is associated with persistence of organism on indwelling medical devices [4]. Bap (biofilm-associated protein) is involved in biofilm formation [4]. AbOmpA plays multiple roles in bacterial pathogenesis, including adherence to host cells, induction of cell death, and serum resistance [5–7]. In *A. baumannii*, several proteins potentially play roles in adherence or biofilm formation, for example: Ata (*Acinetobacter* trimeric autotransporter) acts as adhesive material [8]; beta-lactamase PER-1 probably has correlated with the capacity to form biofilm and to adhere to epithelial cells [9]; filamentous hemagglutinin adhesion B (FhaB) is associated with biofilm formation [10], and phosphorylcholine (ChoP) interacts with host cells. [11].

Recently, it has been shown that *A. baumannii* harbors a wide repertoire of virulence factors, which are common to the genomes of most strains, but are differentially expressed according to each individual strain [6]. The purpose of this study was to perform molecular detection and in vitro and in vivo evaluations of virulence factors involved in serum resistance, biofilm formation, and cell attachment;

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additionally, clonal relatedness among MDR isolates of *A. baumannii* strains was analyzed.

Materials and Methods

Multilocus Sequence Typing (MLST)

In this study, 50 nonduplicate clinical MDR isolates of *A. baumannii* were considered randomly as previously described [12]. MLST was performed using Pasteur's scheme as previously described <http://pubmlst.org/abaumannii/>. The nucleotide sequences and allele profiles of amplified genes (*fusA*, *gltA*, *pyrG*, *recA*, *cpn60*, *rplB*, and *rpoB*) were compared in <http://pubmlst.org/abaumannii/> to identify the allele numbers and the sequence types (STs).

Serum-Resistance Assay

Serum resistance assay was performed based on a modified protocol as described in the study by Antunes et al. [13]. In brief, bacteria were cultured overnight on Muller Hinton agar. The next day, a single colony was inoculated in BHI broth until OD_{600nm} reached values in the range of 0.08–0.13. Each strain of *A. baumannii* was separately suspended in 40% normal human serum (NHS) and heat-inactivated serum used as controls. Following 3-h incubation at 37 °C, bacterial isolates were serially diluted in PBS and cultured on Blood agar to determine colony counts (CFU/ml). *A. baumannii* strain AB-44 [14] and *E. coli* ATCC 25922 were used as highly serum-resistant and highly serum-susceptible

strains, respectively. The CFU/ml of bacteria in heat-inactivated serums were divided into CFU/ml of bacteria in 40% NHS. The ratios of 0.6–1, <0.6–0.4, and <0.4 were considered as serum resistant (R), moderate (M), and susceptible (S), respectively.

Biofilm-Formation Test

Biofilm formation was determined using crystal violet method. In brief, bacterial suspensions in LB broth with 0.5% glucose were aliquoted in 96-well polystyrene microtiter plate and incubated at 37 °C for 72 h. After three washes with PBS, the crystal violet (0.1% W/V) bound to the adherent cells was re-solubilized in ethanol/acetone 80:10 (V/V) and quantified in OD_{570nm} using ELISA reader (BioTek Synergy4, Winooski, USA). The detailed method and biofilm capacities of the test strains were categorized as previously defined [15].

Detection of Virulence Genes

PCR assays were carried out for detection of six virulence genes including *choP*, *ata*, *bap*, *fhaB*, *bla_{PER-1}*, and *ompA* using a set of specific primers and different annealing temperatures as shown in Table 1. The *ompA* genes in all isolates were also sequenced. The nucleotide sequences were compared with GenBank database using BLAST tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Moreover, virulence genes on the selected genomes of various STs were detected

Table 1 Primer sequences used in this study

Primer name	Primer sequence (5'→3')	T _a (°C)	Amplicon size (bp)	References
<i>choP</i>	F: GCAGCGTACTCCATTCGTTA R: TGTAGCCCAAAGAAACCGTATGAT	56	344	This study
<i>ata</i>	F: ATCCAACAACCGAAGCGAATCA R: ACCACCTACAGCAGCAGCAT	58	398	This study
<i>bap</i>	F: ATGCTGAGATACAAATTATTGCCAAGGATAATC R: AGGTGCTGAAGAATCATCATYAC	55	561	[25]
<i>fhaB</i>	F: ACTCAGCAGGTACAGTCGTTCC R: GCCTTGTCAGCATTGTTCCAGA	57	1435	This study
<i>bla_{PER-1}</i>	F: TGCCTCGACGCTACTGATGGT R: GCTCTGGTCTGTGGTGGTTTC	60	644	This study
<i>ompA</i>	F: ACTATGCTTGTTGCTGCTCCAT R: ACAGTACGGCTACCAGTGATTG	57	995	This study
<i>ompA</i> expression <i>ompA</i> TaqMan probe	F: AGCTCTTGCTGGCTTAAACG R: GAGCAACTGGAGTTGGTTCA [FAM]CAGCAGGCTTCAAGTGACCACCA[TAM]	54	95	[26]
rRNA expression rRNA TaqMan probe	F: GTAGCGGTGAAATGCGTAGA R: CTTTCGTACCTCAGCGTCAG [FAM]CGAAGGCAGCCATCTGGCCT[TAM]	54	85	[26]

using BLAST tool in ENSEMBL server (<http://bacteria.ensembl.org/Multi/Tools/Blast?db=core>).

***ompA* Expression**

Eighteen isolates with strong serum-resistant capability were selected and analyzed for expression of the *ompA* gene. In brief, the bacteria were cultured in Luria–Bertani broth until OD reached 0.6 in 600 nm. The bacterial RNA was extracted using AccuZol kit (Bioneer, Seoul, Korea). Relative quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was performed using a LightCycler[®] 96 (Roche Diagnostics, Mannheim, Germany) according to the protocol as previously described [16]. The primers and TaqMan probes were used as described in Table 1. The experiment was repeated in triplicate assay. The negative controls were TaqMan Real-Time PCR Master Mix (ThermoFisher, USA) without reverse-transcriptase that confirmed the absence of DNA contamination in the samples. Relative gene expression was determined using the $2^{-\Delta\Delta C_t}$ method. The 16S rRNA gene was used as a reference gene for normalization of gene expression, and *A. baumannii* ATCC 19606 was used as a reference sample.

Spleen Colony Counts and Serum IL-6 Measurements

In this study, a murine model of disseminated peritoneal sepsis was used as described elsewhere [17]. In brief, bacteria were cultured overnight on Mueller-Hinton agar. The next day, a single colony was inoculated in BHI broth until OD_{600nm} reached to 0.08–0.13. 100 μ l of bacterial suspensions (including ~ 500 colony-forming units) was mixed with 100 μ l of 10% (V/V) porcine stomach mucin (Sigma, USA). This suspension was injected intraperitoneally into 6–8 week-old C57BL/6 mice. The spleens aseptically were extracted followed by anesthetization of mice. The spleen suspensions were cultured on blood agar plates. After 24 h, the colonies were counted. *A. baumannii* ATCC 19606 was used as a reference strain. Moreover, for detection of bacteria in bloodstream, 100 μ l heart's blood was cultured on blood agar plates. The bacteremia of the selected strains was reported according to CFU/ml (Bacteremia index). Serum levels of IL-6 in mice were determined using IL-6 ELISA Ready-SET-Go kit (eBioscience, San Diego, Calif., USA) and measured using ELISA reader (BioTek Synergy4, Winooski, USA). Three mice were considered for each isolate. Moreover, spleen extractions, heart's blood, and serum collections were obtained simultaneously 18 h post-intraperitoneal injections.

Statistical Analyses

The correlation between two ordinal variables was tested using Spearman's rank correlation ρ test. Moreover, the in vivo results of strains were compared by One-Way ANOVA or unpaired *t* test. Statistical analyses and graphs were performed using GraphPad Prism version 6.01 (GraphPad Software Inc., San Diego California, USA). In all experiments, $P < 0.05$ was considered statistically significant.

Ethical Statement

This project was done based on ethical guidelines as previously approved by the Pasteur institute of Iran (Ethics No.: IR.PII.REC.1397.015).

Results

MLST Results

The sequences analysis revealed the presence of three sequence types among isolates including ST2 (64%, 32/50), ST513 (30%, 15/50), and ST1 (6%, 3/50) as shown in Table 3. The allele numbers of seven housekeeping genes among reported STs are as follows: ST1 (1–1–1–1–5–1–1), ST2 (2–2–2–2–2–2–2), and ST513 (56–3–55–2–9–4–14).

Serum Resistance and Biofilm Formation

The serum-resistance capacity among isolates was graded as 18% (9/50) sensitive, 28% (14/50) moderate, and 54% (27/50) resistant (Table 3). The biofilm-formation capacity among isolates was graded as 22% (11/50) weak, 66% (33/50) moderate, and 12% (6/50) strong (Table 3). No significant positive or negative correlation was observed in Spearman's rank correlation (ρ) test between serum resistance and biofilm formation ($P > 0.05$).

Detection of Virulence Genes

PCR assays and in silico analysis revealed that patterns of virulence genes were strongly related to Pasteur's ST as shown in Tables 2 and 3. The patterns of virulence genes including *choP*, *ata*, *bap*, *fhaB*, *bla*_{PER-1}, and *ompA* types among ST2, ST513, and ST1 were (–, +, +, +, – and type I), (–, –, +, –, + and type II), and (+, –, +, –, – and type II), respectively. DNA sequences of *ompA* type I and type II were deposited in GenBank database under accession numbers KP271243 and KP271242, respectively. The naming of type I and II is contractual.

Table 2 The clinical information, in silico detection of virulence factors, and allele types of *ompA* among different sequence types (STs) of selected *A. baumannii* strains

No.	Strain	Country	Year	Source	Clonal complex	Pasteur's MLST sequence type	Virulence factors					
							<i>choP</i>	<i>ata</i>	<i>bap</i>	<i>fhaB</i>	<i>blaPER-1</i>	<i>ompA</i> type
1	NIPH 527	Netherlands	1984	U	CC1	ST1	+	-	+	-	-	II
2	IS-235	Iraq	2008	B	CC1	ST1	+	-	+	-	-	II
3	AYE	France	2001	U	CC1	ST1	+	-	+	-	-	II
4	AB5075	USA	2008	-	CC1	ST1	+	-	+	-	-	II
5	Naval-83	USA	2003	B	CC1	ST20	+	-	+	-	-	II
6	ACICU	Italy	2005	CSF	CC2	ST2	-	+	+	+	-	I
7	MDR-ZJ06	China	2006	B	CC2	ST2	-	+	+	+	-	I
8	MDR-TJ	China	-	-	CC2	ST2	-	+	+	+	-	I
9	BJAB0868	China	-	AF	CC2	ST2	-	+	+	+	-	I
10	6014059	USA	-	S	CC2	ST2	-	+	+	+	-	I
11	NCGM 237	Japan	2012	-	CC2	ST2	-	+	+	+	-	I
12	NIPH 1362	Czech	2000	URT	CC2	ST47	-	+	+	+	-	I
13	IS-143	Iraq	-	W	CC2	ST414	-	+	+	+	-	I
14	ABNIH3	USA	-	-	CC2	ST415	-	+	+	+	-	I
15	16553_8	USA	-	-	CC2	ST415	-	+	+	+	-	I
16	UH8907	USA	-	-	CC2	ST524	-	+	+	+	-	I
17	Naval-13	USA	2006	W	CC3	ST3	+	+	+	-	-	II
18	NIPH 1669	Netherlands	1997	B	CC3	ST3	+	+	+	-	-	II
19	IS-123	Iraq	2009	W	CC3	ST3	+	+	+	-	-	II
20	OIFC109	USA	2003	-	CC3	ST3	+	+	+	-	-	II
21	Naval-81	USA	2006	-	CC3	ST3	+	+	+	-	-	II
22	OIFC137	USA	2003	-	CC3	ST3	+	+	+	-	-	II
23	WC-A-694	USA	2008	-	CC3	ST3	+	+	+	-	-	II
24	WC-692	Iraq	2008	S	Singleton	ST513	-	-	+	-	+	II
25	ATCC19606	USA	-	U	ND	ST52	+	+	+	+	-	non-I&II
26	ATCC17978	France	1951	M	ND	ST77	+	+	+	-	-	non-I&II
27	D1279779	Australia	2009	B	ND	ST267	-	+	-	-	-	non-I&II
28	SDF	France	2004	HBL	Singleton	ST17	-	-	-	-	-	non-I&II

B blood, U urine, CSF cerebrospinal fluid, AF abdominal fluid, S skin, RS rectal swab, URT upper respiratory tract, W wound, ST stool, IVC intravenous catheter, SP sputum, M meningitis, HBL human body louse, ND means not determined

Spleen Colonization and Determination of Bacterial Loads in Bloodstream

We determined the bacterial loads in spleen to estimate proliferation capacity of strains in mouse model. Moreover, the bacterial loads in bloodstream (calculated as 'bacteremia index', BI = CFU/ml of bacterial loads in bloodstream obtained 18 h' post-injection) were measured for all mice [18]. The average of Log_{10} CFU/g \pm SD of bacteria recovered from spleens of C57/BL6 mice is shown in Fig. 1. The range of Log_{10} CFU/g was from 4.10 (AB-40 strain) to 7.72 (AB-33 strain). One-Way ANOVA test showed that the Log_{10} CFU/g results of strains (except AB-40, AB-38, and AB-15) had significant differences with reference strain ($P < 0.05$). Moreover, the bacteremia was confirmed in 9/18 of strains (Table 3).

Levels of IL-6 in Serum

It seems that the serum level of IL-6 can be considered as a serum marker characterizing the invasiveness of *A. baumannii* strains [19]. Therefore, we measured this pro-inflammatory cytokine in mice serum. We selected two groups of MDR isolates based on spreading in bloodstream in C57/BL mice. Group A including AB-38, AB-40, AB42, AB-47, AB-30, AB-1, AB-5, AB-45, and AB-43 (with spreading capability in bloodstream); and group B including AB-32, AB-12, AB-11, AB-15, AB-3, AB-19, AB-46, AB-41, and AB-33 (without spreading capability in bloodstream) are shown in Fig. 1. The average levels of IL-6 \pm SD among groups A and B were 229.38 ± 24.22 and 145.15 ± 12.04 pg/ml, respectively. Unpaired *t* test showed

Table 3 The clinical information, prevalence of virulence factors, serum resistance, biofilm formation, and in vivo study of MDR *A. baumannii* isolated from clinical settings

No.	Strain	Hospital	Source	Pasteur's ST	choP	ata	bap	fhaB	bl ₄ PER1	ompA type	Serum resistance	Biofilm formation	ompA expression (mean of fold change)	Bacteremia index (CFU/ml)
1	AB-1	L	TA	ST2	-	+	+	+	-	I	R	++	1.57	800
2	AB-2	L	TA	ST2	-	+	+	+	-	I	S	+++	ND	ND
3	AB-3	L	U	ST2	-	+	+	+	-	I	R	+	0.93	-
4	AB-4	L	TA	ST2	-	+	+	+	-	I	M	++	ND	ND
5	AB-5	L	TA	ST2	-	+	+	+	-	I	R	++	1.501	1600
6	AB-6	L	U	ST2	-	+	+	+	-	I	M	++	ND	ND
7	AB-7	L	TA	ST2	-	+	+	+	-	I	S	++	ND	ND
8	AB-8	L	S	ST2	-	+	+	+	-	I	S	+++	ND	ND
9	AB-9	L	C	ST2	-	+	+	+	-	I	M	++	ND	ND
10	AB-10	M	W	ST2	-	+	+	+	-	I	R	++	ND	ND
11	AB-11	M	W	ST2	-	+	+	+	-	I	R	++	1.09	-
12	AB-12	E	U	ST2	-	+	+	+	-	I	R	++	1.07	-
13	AB-13	E	U	ST2	-	+	+	+	-	I	M	++	ND	ND
14	AB-14	L	A	ST2	-	+	+	+	-	I	S	++	ND	ND
15	AB-15	L	U	ST2	-	+	+	+	-	I	R	+	1.25	-
16	AB-16	K	BAL	ST2	-	+	+	+	-	I	S	+	ND	ND
17	AB-17	K	S	ST2	-	+	+	+	-	I	R	++	ND	ND
18	AB-18	L	S	ST2	-	+	+	+	-	I	S	+++	ND	ND
19	AB-19	E	S	ST2	-	+	+	+	-	I	R	++	1.083	-
20	AB-20	E	S	ST2	-	+	+	+	-	I	R	++	ND	ND
21	AB-21	L	S	ST2	-	+	+	+	-	I	S	++	ND	ND
22	AB-22	L	TA	ST2	-	+	+	+	-	I	M	++	ND	ND
23	AB-23	L	TA	ST2	-	+	+	+	-	I	M	++	ND	ND
24	AB-24	E	U	ST2	-	+	+	+	-	I	S	+	ND	ND
25	AB-25	L	TA	ST2	-	+	+	+	-	I	R	++	ND	ND
26	AB-26	L	B	ST2	-	+	+	+	-	I	R	++	ND	ND
27	AB-27	L	TA	ST2	-	+	+	+	-	I	R	++	ND	ND
28	AB-28	L	TA	ST2	-	+	+	+	-	I	R	+	ND	ND
29	AB-29	K	S	ST2	-	+	+	+	-	I	M	+++	ND	ND
30	AB-30	K	CSF	ST2	-	+	+	+	-	I	R	++	1.737	3100
31	AB-31	E	U	ST2	-	+	+	+	-	I	R	++	ND	ND
32	AB-32	E	U	ST2	-	+	+	+	-	I	R	+	0.91	-
33	AB-33	L	B	ST513	-	-	+	-	+	II	R	++	1.028	-
34	AB-34	L	S	ST513	-	-	+	-	+	II	M	+++	ND	ND

Table 3 (continued)

No.	Strain	Hospital	Source	Pasteur's ST	choP	ata	bap	fhaB	bl ₄ PER1	ompA type	Serum resistance	Biofilm formation	ompA expression (mean of fold change)	Bacteremia index (CFU/ml)
35	AB-35	L	TA	ST513	-	-	+	-	+	II	S	++	ND	ND
36	AB-36	L	U	ST513	-	-	+	-	+	II	M	+	ND	ND
37	AB-37	L	TA	ST513	-	-	+	-	+	II	M	++	ND	ND
38	AB-38	S	C	ST513	-	-	+	-	+	II	R	++	2.005	2300
39	AB-39	K	BAL	ST513	-	-	+	-	+	II	M	+	ND	ND
40	AB-40	K	C	ST513	-	-	+	-	+	II	R	++	1.671	2100
41	AB-41	K	BAL	ST513	-	-	+	-	+	II	R	++	0.823	-
42	AB-42	K	B	ST513	-	-	+	-	+	II	R	++	1.701	3300
43	AL-43	L	SF	ST513	-	-	+	-	+	II	R	++	1.52	1800
44	AB-44	L	U	ST513	-	-	+	-	+	II	M	++	ND	ND
45	AB-45	L	TA	ST513	-	-	+	-	+	II	R	+	1.954	800
46	AB-46	L	B	ST513	-	-	+	-	+	II	R	+	1.013	-
47	AB-47	L	S	ST513	-	-	+	-	+	II	R	+++	1.441	600
48	AB-48	L	TA	ST1	+	-	+	-	-	II	M	++	ND	ND
49	AB-49	M	W	ST1	+	-	+	-	-	II	R	++	ND	ND
50	AB-50	M	W	ST1	+	-	+	-	-	II	M	+	ND	ND

TA tracheal aspirate, U urine, S sputum, B blood, C catheter, W wound secretions, A abscess, BAL bronchoalveolar lavage, SF synovial fluid, CSF cerebrospinal fluid, Bacteremia index BI = CFU/ml of bacterial loads in bloodstream obtained 18 h post-injection, ND means not determined

Fig. 1 The mean of Log_{10} CFU/g \pm SD among MDR isolates recovered from spleens of C57/BL6 mice. There is a wide range of Log_{10} CFU/g from 4.10 (AB40 strain) to 7.72 (AB33strain). The One-Way ANOVA test showed the Log_{10} CFU/g of MDR strains had significant differences with reference strain (P -value < 0.05). *A. baumannii* ATCC 19606 was used as reference strain

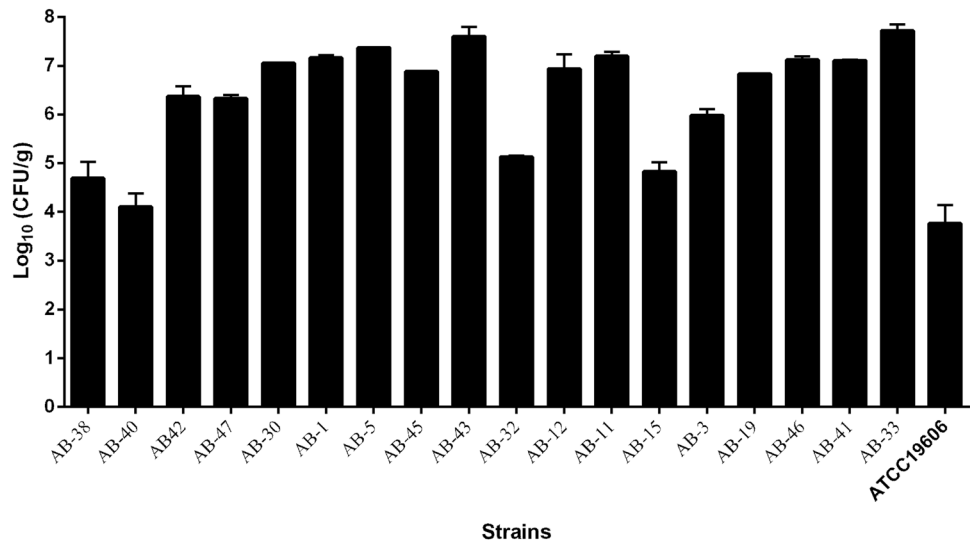
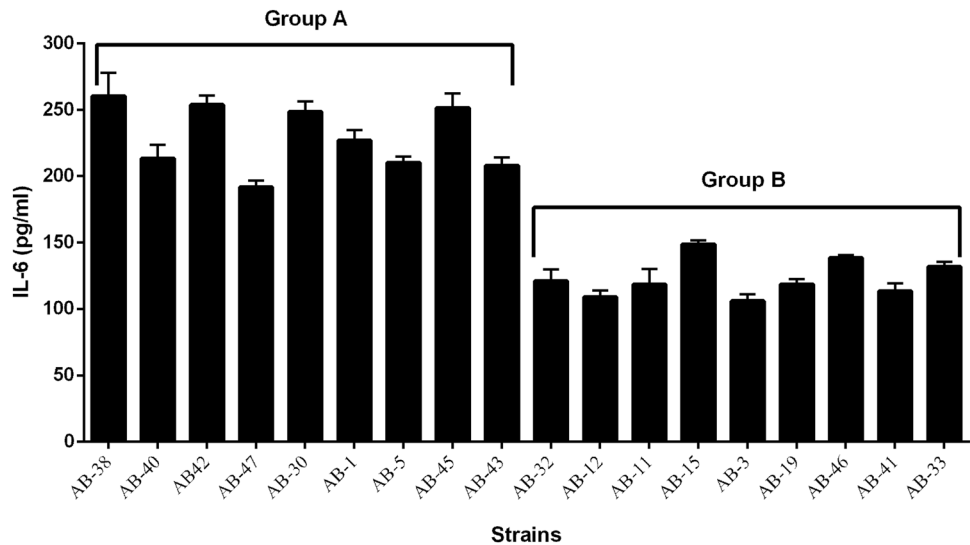


Fig. 2 The amounts of IL-6 (pg/ml) in serum of C57/BL6 mice. There are two groups A (with capability of bacteremia in mice) and B (without capability of bacteremia in mice) among clinical MDR isolates. Unpaired t test showed that the levels of IL-6 between groups A and B had a significant difference (P -value < 0.05)



that the amounts of IL-6 between groups A and B had a significant difference (P value < 0.05). See Fig. 2.

***ompA* Expression**

The means of fold-changes in *ompA* expressions among MDR isolates have been shown in Table 3. The range of expression was 0.823–2.005. Unpaired t test results showed that groups A and B were statistically significant ($P < 0.001$). However, there was no statistically significant difference between *ompA* expression of ST2 and ST513 ($P > 0.05$).

Discussion

Acinetobacter baumannii causes a variety of nosocomial infections with a particular ability to survive in hostile environments and capture numerous antimicrobial

resistance elements [20]. Data have shown that majority of outbreaks reported around the world are associated with MLST clonal complexes CC92/CC2 (Oxford/Pasteur), corresponding to Pasteur sequence types including ST2, ST47, ST414, ST415, and ST524 (Table 2). Our findings are consistent with previous results. Moreover, ST513 as a singleton and endemic clone in Middle East is closely related to ST323 (56–3–55–2–5–1–14) as previously described in Iran [21].

It can be argued that clinical isolates recovered from exterior fluids including urine, sputum, etc., need adhesion factors involved in attachment and biofilm formation. On the other hand, isolates recovered from interior fluids including blood, synovia fluid, and cerebrospinal fluid need serum-resistance capability to establish their infections. In our study, isolates recovered from sputum mostly showed a high capacity to form a biofilm. However, all isolates

recovered from interior fluids showed a high serum-resistance capacity.

In the present study, in silico comparative analysis of bacterial genomes extracted from ENSEMBL database revealed the presence or the absence of *choP*, *ata*, *bap*, *fhaB*, and *bla_{PER-1}* in genomes as well as variations in *ompA* sequences. On the other hand, the rest of virulence genes including *csuA/BABCDE*, *pgaABCD*, *pbpG*, *ptk*, *epsA*, *pld*, *entA*, *plc1*, *nfuA*, *CipA*, *lpsB*, *Omp33-36*, *omp22*, *carO*, *tuf*, *abeD*, *gacS*, *paaE*, *surA1*, *uspA*, and *recA* were mostly present in all *Acinetobacter* spp. genomes except SDF as a nonpathogenic strain. Comparative in silico analysis of surface-exposed virulence factors of *A. baumannii* has shown that the known or putative virulence determinants were restricted to specific clonal lineages suggesting that these virulence determinants may be crucial for overcoming these successful clones [22]. Our PCR assays and in silico analysis revealed that the pattern of virulence genes was strongly related to Pasteur's ST as shown in Tables 2 and 3. However, as was proved earlier, the virulence-associated phenotypes cannot be associated with clonal relatedness, or vice versa.

AbOmpA as a multifaceted protein was recently shown to be a major virulence factor [23]. Paired sequence alignment of type I and type II of AbOmpA showed there are sequence variations in extracellular loops (data not shown). It seems that these sequence variations render a different virulence capability among clinical isolates. qRT-PCR analysis showed that sepsis-producing strains overexpress *ompA* significantly with respect to the level of IL-6 in bloodstream of mice model. These results showed that high expression of *ompA* possibly plays an important role in the infectious capacity caused by *A. baumannii*. Moreover, it is noteworthy that the difference in *ompA* expressions observed between MDR isolates is clonally independent.

In sepsis murine model after the peritoneal injection, the acute inflammatory response is triggered, and subsequently, innate immune cells infiltrated in peritoneal cavity. The bacteria are phagocytosed and transported to spleen for antigen presentation [24]. Our study showed that all 18 selected strains proliferated in the spleen of C57/BL6 mice. On the other hand, only 9/18 of bacteria were detected in bloodstream of mice model. These findings suggest that the bacteria possibly have different proliferation capabilities and bloodstream invasions. Interestingly, AB-38 and AB-40 strains were released in bloodstream efficiently, while they had low Log₁₀ CFU/g scores (4.69 and 4.1, respectively). Moreover, the strains which caused bacteremia in mice had higher and significant levels of serum IL-6 compared to isolates without bacteremia in mice model. This finding showed that during spleen transportation of the strains which had strong invasiveness, the larger population of them evades from host immune system and released into bloodstream.

The present study showed that indistinguishable clones have the same pattern of virulence genes, while, the virulence traits and expressions of *ompA* were clonally independent. Recently, some studies have focused on transcriptome and expression of virulence apparatus to clarify different virulence capability among clinical isolates [25, 26]. However, drawing a complete picture of the host–pathogen interaction associated with *A. baumannii* remains to be explored and elucidated.

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Compliance with Ethical Standards

Conflict of interest The authors have no conflicts of interest to declare.

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