

Title: Biofilm formation is not associated with worse outcome in *Acinetobacter baumannii* bacteraemic pneumonia

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Supplementary information

Supplementary Table S1. Use of antimicrobials and the 28-day mortality rates in patients with bacteraemic pneumonia caused by non-biofilm-forming *Acinetobacter baumannii*.

Main agents used	APACHE II score,		Patients, no. (%)			
	Patients, no.	Median (interquartile range)	Appropriate antimicrobial therapy	Combination antimicrobial therapy	28-Day non-survivors	28-Day survivors
Anti-pseudomonal penicillins ^a	24	25.46 (19.25–30)	11 (45.8)	4 (16.7)	14 (58.3)	10 (41.7)
Anti-pseudomonal cephalosporins ^b	29	26.62 (21-33)	4 (13.8)	9 (31)	15 (51.7)	14 (48.3)
Anti-pseudomonal fluoroquinolones ^c	8	29.25 (16.5-39.75)	1 (12.5)	3 (37.5)	4 (50)	4 (50)
Anti-pseudomonal carbapenems ^d	65	26.63 (21.5-32)	19 (29.2)	14 (21.5)	37 (56.9)	28 (43.1)
Ampicillin/sulbactam or sulbactam	19	28.16 (22-39)	6 (31.6)	10 (52.6)	11 (57.9)	8 (42.1)
Non-antipseudomonal β -lactamases ^e	20	29.2 (19-38.75)	8 (40)	1 (5)	11 (55)	9 (45)

* Data are the median (interquartile range) for continuous variables and number of cases (%) for categorical variables. APACHE II = Acute Physiologic and Chronic Health Evaluation II.

^apiperacillin, piperacillin/tazobactam, and ticarcillin/clavulanate.

^bcefoperazone, ceftazidime, cefepime, and ceftazidime/ceftazidime.

^cciprofloxacin and levofloxacin.

^dimipenem and meropenem.

^epenicillin, amoxicillin/clavulanate, ceftazidime, cefuroxime, cefotaxime, cefmetazole, and flomoxef.

Supplementary Table S2. The minimum inhibitory concentration (MIC) and minimum biofilm eradication concentration (MBEC) of imipenem and meropenem against 10 *Acinetobacter baumannii* isolates.

Drugs		Imipenem		Meropenem	
Strains	Pulsotype	MIC (mg/L)	MBEC (mg/L)	MIC (mg/L)	MBEC (mg/L)
13	P4	64	>2048	128	>2048
41	P5	4	>2048	2	>2048
93	P11	8	>2048	16	>2048
99	P14	64	>2048	64	>2048
102	P15	16	>2048	32	>2048
137	P31	0.25	>1024	0.25	>1024
146	P36	0.5	>1024	0.25	>1024
156	P43	64	>2048	0.25	>1024
177	P60	1	1024	4	>2048
190	P68	4	>2048	32	>2048

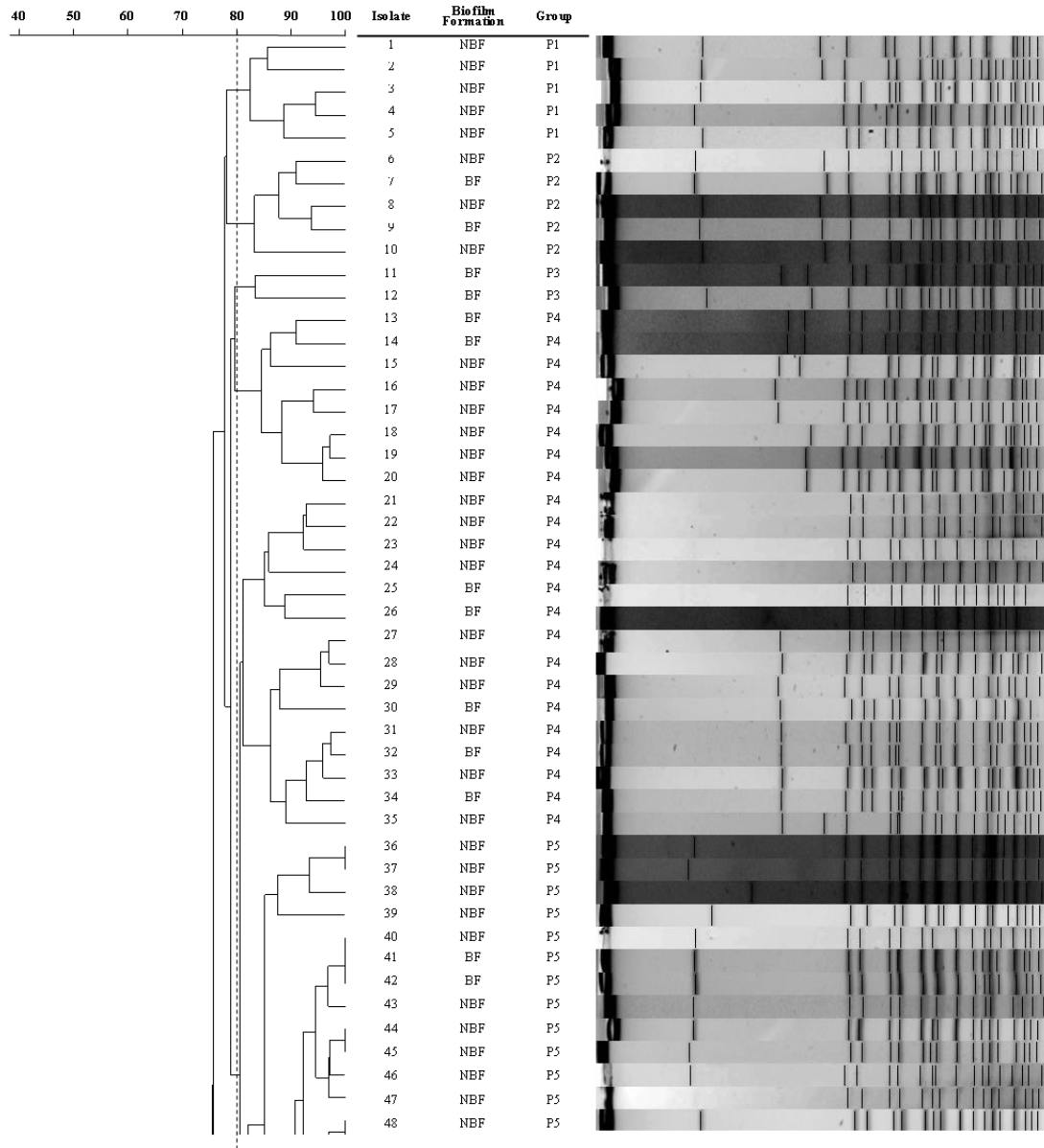
* Values represent the mode of at least 3 biological replicates.

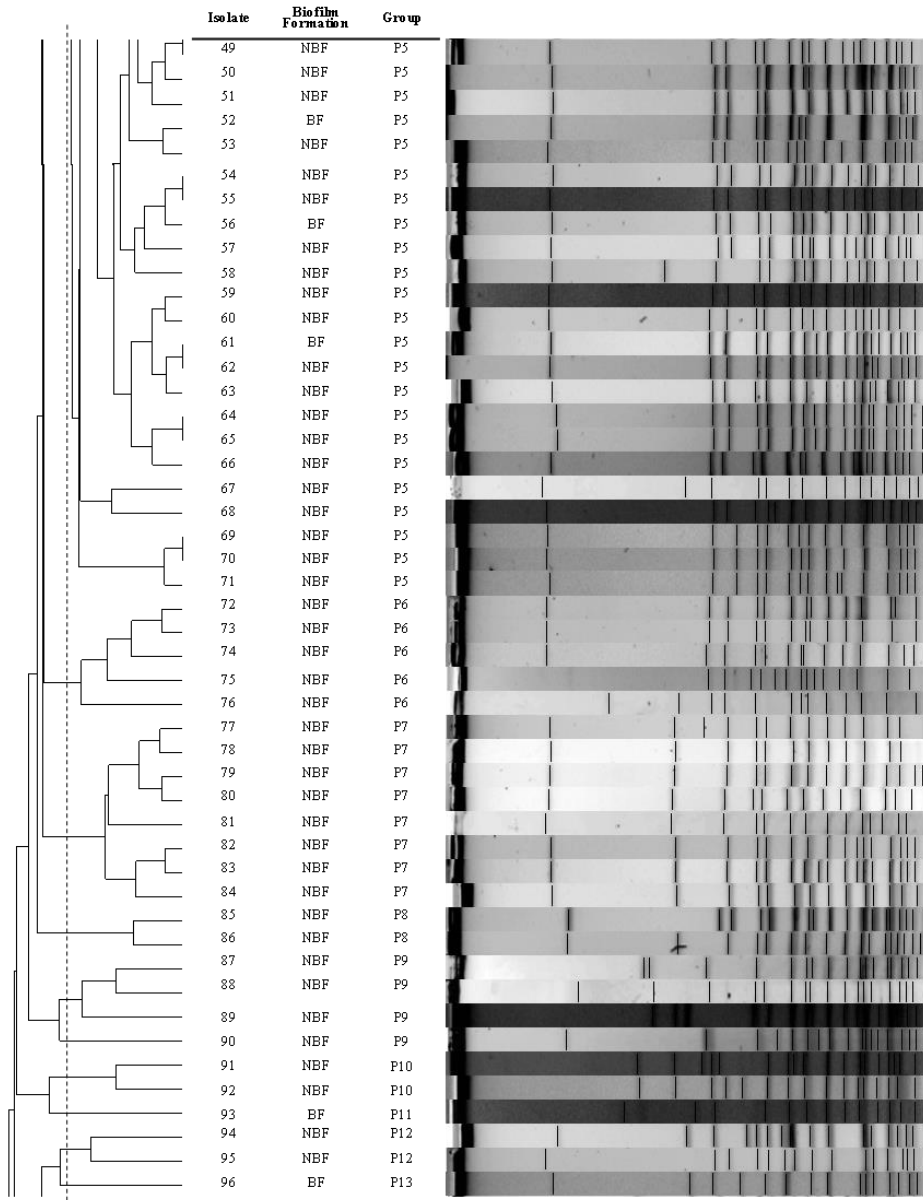
Supplementary Table S3. Biofilm-associated genes of selected *Acinetobacter baumannii* isolates.

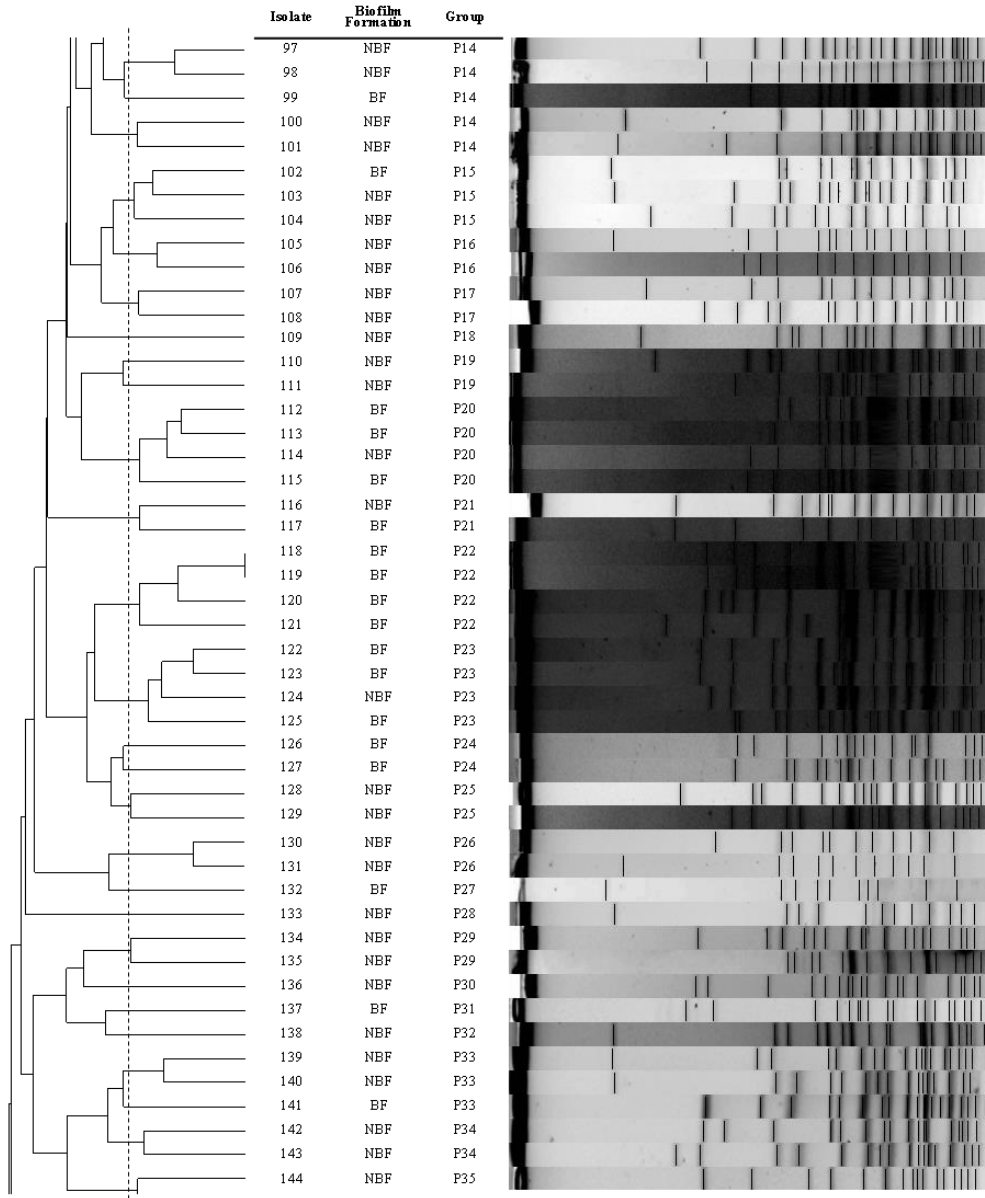
Isolates	Pulsotype	<i>BfmS</i>	<i>AbaI</i>	<i>Bap1</i>	<i>Bap2</i>	<i>pgaABCD</i>	<i>CsuAB/ABCDE</i>					
							<i>CsuA</i>	<i>CsuB</i>	<i>CsuC</i>	<i>CsuD</i>	<i>CsuE</i>	<i>CsuA/B</i>
Biofilm-forming isolates (n=9)												
32	P4	+	+	+	+	+	+	+	+	+	+	+
102	P15	+	+	+	+	+	+	+	+	+	+	+
145	P35	+	+	+	+	+	+	+	+	+	+	+
154	P41	+	+	+	+	+	+	+	+	+	+	+
156	P43	+	+	-	-	+	+	+	+	+	+	+
160	P46	+	+	-	-	+	+	+	+	+	+	+
171	P56	+	+	+	+	+	+	+	+	+	+	+
177	P60	+	+	+	+	+	+	+	+	+	+	+
181	P61	+	+	+	+	+	+	+	+	+	+	+
Total, no. (%)		9 (100%)	9 (100%)	7(77.8%)	7(77.8%)	9 (100%)	9 (100%)	9 (100%)	9 (100%)	9 (100%)	9 (100%)	9 (100%)
Non-biofilm-forming isolates (n=30)												
3	P1	+	+	+	+	+	+	+	+	+	+	+
6	P2	+	+	+	+	+	+	+	+	+	+	+
21	P4	+	+	+	+	+	+	+	+	+	+	+
49	P5	+	+	+	+	+	+	+	+	+	+	+
74	P6	+	+	+	+	+	+	+	+	+	+	+
83	P7	+	+	+	+	+	+	+	+	+	+	+
90	P9	+	+	+	+	+	+	+	+	+	+	+
94	P12	+	+	+	+	+	+	+	+	+	+	+
100	P14	+	+	+	+	+	-	-	-	-	-	-
103	P15	+	+	+	+	+	+	+	+	+	+	+
105	P16	+	+	+	+	+	+	+	+	+	+	+
107	P17	+	+	+	+	+	+	+	+	+	+	+

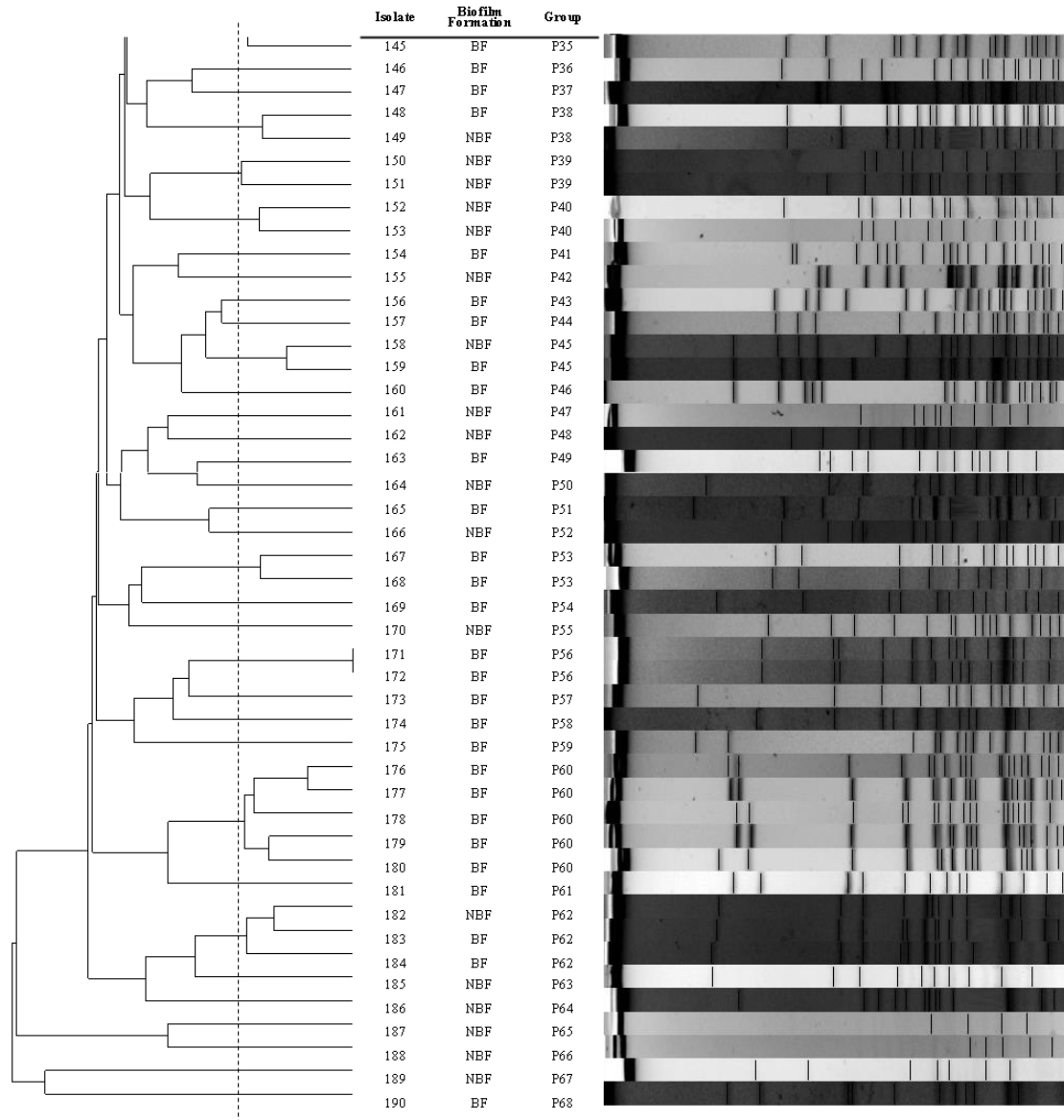
114	P20	+	+	+	+	+	+	+	+	+	+	+	+
124	P23	+	+	+	+	+	+	+	+	+	+	+	+
129	P25	+	+	-	-	+	-	-	+	+	+	+	+
131	P26	+	+	+	+	+	+	+	+	+	+	+	+
133	P28	+	+	-	+	+	+	+	+	+	+	+	+
135	P29	+	+	+	+	+	-	-	-	-	-	-	-
140	P33	+	+	+	+	+	+	+	+	+	+	+	+
142	P34	+	+	+	+	+	+	+	+	+	+	+	+
144	P35	+	+	+	+	+	+	+	+	+	+	+	+
152	P40	+	+	+	+	+	+	+	+	+	+	+	+
158	P45	+	+	+	+	+	+	+	+	+	+	+	+
161	P47	+	+	+	+	+	+	+	+	+	+	+	+
164	P50	+	+	+	+	+	+	+	+	+	+	+	+
166	P52	+	+	+	+	+	+	+	+	+	+	+	+
170	P55	+	+	+	+	+	+	+	+	+	+	+	+
182	P62	+	+	+	+	+	+	-	+	+	+	+	+
186	P64	+	+	+	+	+	-	-	-	-	-	-	-
187	P65	+	+	+	+	+	+	+	+	+	+	+	+
Total, no. (%)		30	30	28	29	30 (100%)	26	26	27	27	27	27	27
		(100%)	(100%)	(93.3%)	(96.7%)		(86.7)	(86.7)	(90%)	(90%)	(90%)	(90%)	(90%)

Supplementary Fig S1. Pulsed-field gel electrophoresis of 190 isolates of *Acinetobacter baumannii* showed two major clones, pulsotype P4 and P5. BF = biofilm-formation, NBF = non-biofilm-formation.

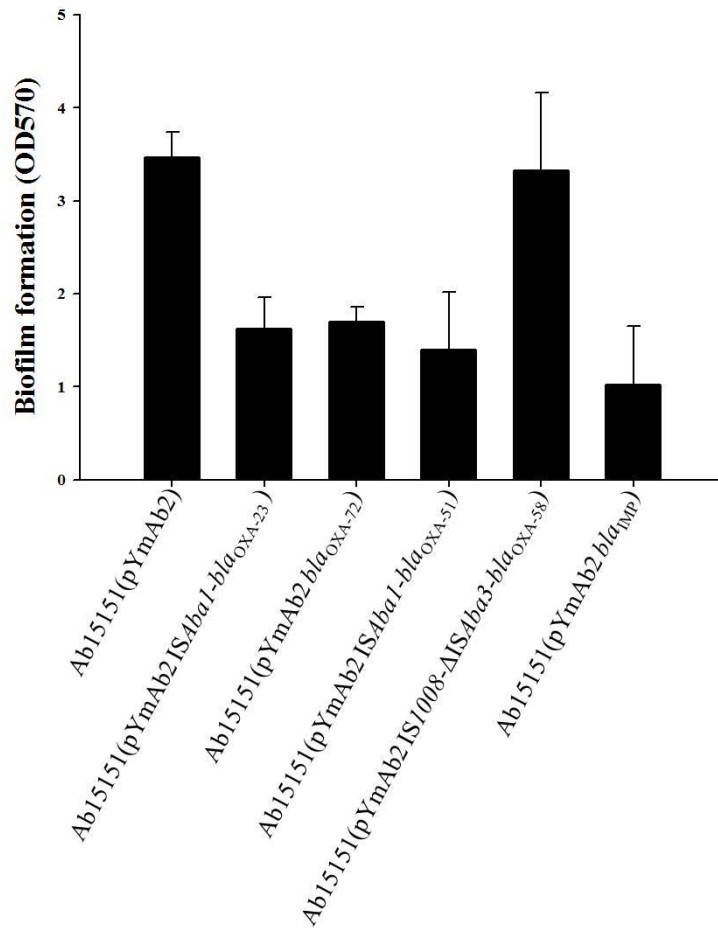






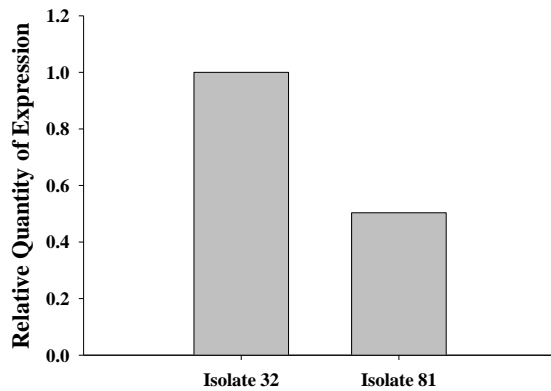


Supplementary Fig S2. Carbapenem-resistant transformants harboring various carbapenemase genes produced less biofilm at 48 h than the *Acinetobacter baumannii* ATCC 15151(Ab15151) carrying an empty vector.

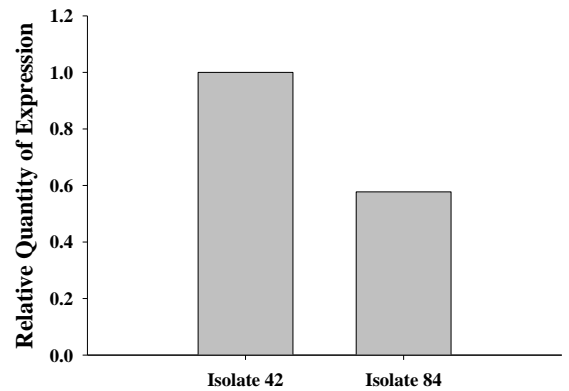


Supplementary Fig S3. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) showed that the expression levels of (a)-(b) *bap1* [fold change 1.99 and 1.73, respectively], (c)-(d) *abaI* [fold change 2.01 and 1.52, respectively], and (e)-(f) *csu A/B* [fold change 8.2 and 5.42, respectively] were higher in the biofilm-forming isolates (isolates 32 and isolates 42) than in the matched non-biofilm-forming isolates (isolates 81 and isolates 84).

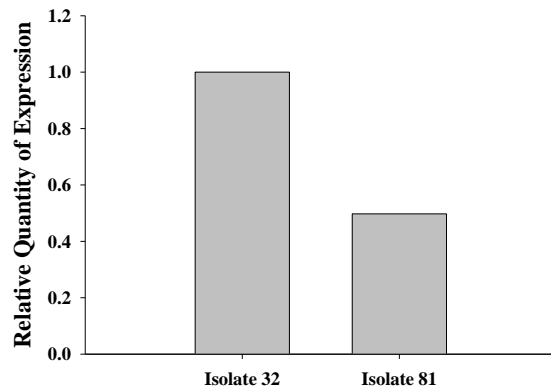
(a) *bap1*



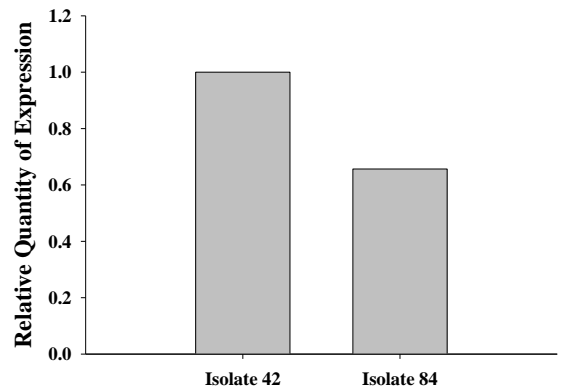
(b) *bap1*



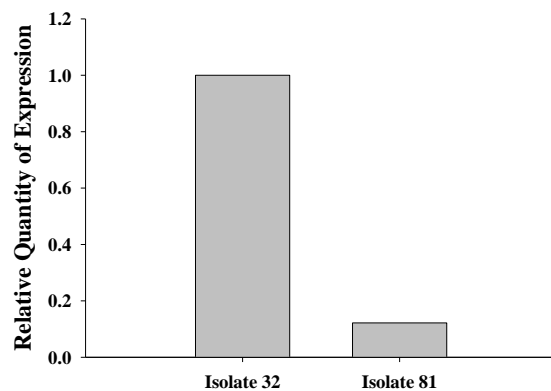
(c) *abaI*



(d) *abaI*



(e) *csuA/B*



(f) *csuA/B*

