

Supplementary materials

Ciprofloxacin- and Levofloxacin-Loaded Nanoparticles Efficiently Suppressed Fluoroquinolone Resistance and Biofilm Formation in *Acinetobacter baumannii*

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Supplementary Table S1. *A. baumannii* isolates identified in this study and their clinical sources.

Isolate Code	Clinical source	Isolate Code	Clinical source	Isolate Code	Clinical source
Ab1	blood	Ab28	blood	Ab55	Wound
Ab2	blood	Ab29	sputum	Ab56	Wound
Ab3	blood	Ab30	sputum	Ab57	Wound
Ab4	blood	Ab31	sputum	Ab58	Wound
Ab5	blood	Ab32	sputum	Ab59	Wound
Ab6	blood	Ab33	sputum	Ab60	Wound
Ab7	blood	Ab34	sputum	Ab61	Wound
Ab8	blood	Ab35	sputum	Ab62	Wound
Ab9	blood	Ab36	sputum	Ab63	Wound
Ab10	blood	Ab37	sputum	Ab64	Wound
Ab11	blood	Ab38	sputum	Ab65	Wound
Ab12	blood	Ab39	sputum	Ab66	Wound
Ab13	blood	Ab40	sputum	Ab67	Wound
Ab14	blood	Ab41	sputum	Ab68	Wound
Ab15	blood	Ab42	sputum	Ab69	Wound
Ab16	blood	Ab43	sputum	Ab70	Wound
Ab17	blood	Ab44	sputum	Ab71	Wound
Ab18	blood	Ab45	sputum	Ab72	Wound
Ab19	blood	Ab46	sputum	Ab73	Wound
Ab20	blood	Ab47	sputum	Ab74	Wound
Ab21	blood	Ab48	sputum	Ab75	Wound
Ab22	blood	Ab49	sputum	Ab76	Wound
Ab23	blood	Ab50	sputum	Ab77	Urine
Ab24	blood	Ab51	sputum	Ab78	Urine
Ab25	blood	Ab52	sputum	Ab79	Urine
Ab26	blood	Ab53	sputum	Ab80	Urine
Ab27	blood	Ab54	sputum		

Supplementary Table S2. Resistance pattern of *A. baumannii* clinical isolates and standard strains *A. baumannii* ATCC 19606 and 17978 to different antimicrobial agents.

Isolate code	Antimicrobial agents												Resistance pattern	
	β-Lactams						Quinolones		Aminoglycosides		Sulfa drug	Tetracyclines		
	CAZ	FEP	CTX	TZP	SAM	IPM	CIP	LEV	AK	CN	SXT	DO	MIN	
Ab1	R	R	R	R	R	R	R	R	S	R	R	R	R	Yes
Ab2	R	R	R	R	R	R	R	R	R	R	R	S	R	Yes
Ab3	R	R	R	R	R	R	R	R	R	R	R	S	S	Yes
Ab4	R	R	R	R	R	R	R	R	R	R	R	S	S	Yes
Ab5	R	R	R	R	R	R	R	R	R	R	R	S	R	Yes
Ab6	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab7	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab8	R	R	R	R	R	R	R	R	R	R	R	R	I	Yes
Ab9	R	R	R	R	R	R	R	R	R	R	R	S	S	Yes
Ab10	R	R	R	R	R	R	R	R	R	R	R	S	S	Yes
Ab11	R	R	R	R	R	R	R	R	R	R	R	S	S	Yes
Ab12	R	R	R	R	R	R	R	R	R	R	R	S	S	Yes
Ab13	R	R	R	R	R	R	R	R	R	R	R	S	S	Yes
Ab14	R	R	R	R	R	R	R	R	R	R	R	S	S	Yes
Ab15	R	R	R	R	R	R	R	R	R	R	R	S	S	Yes
Ab16	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab17	R	R	R	R	R	R	R	R	R	R	I	S	S	Yes
Ab18	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab19	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab20	R	R	R	R	R	R	R	R	R	S	R	S	S	Yes
Ab21	R	R	R	R	R	R	R	R	R	R	R	S	R	Yes
Ab22	R	R	R	R	R	R	R	R	R	R	R	R	I	Yes
Ab23	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab24	R	R	R	R	R	R	R	R	R	S	R	S	S	Yes
Ab25	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab26	R	R	R	R	R	R	R	R	R	R	R	S	S	Yes
Ab27	R	R	R	R	R	R	R	R	R	R	R	S	S	Yes
Ab28	R	R	R	R	R	R	R	R	R	R	R	S	R	Yes
Ab29	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab30	R	R	R	R	R	R	R	R	R	R	R	S	R	Yes
Ab31	R	R	R	R	R	R	R	R	R	R	R	I	R	Yes
Ab32	R	R	R	R	R	R	R	R	R	R	R	S	I	Yes
Ab33	R	R	R	R	R	R	R	R	R	R	R	S	R	Yes
Ab34	R	R	R	R	R	R	R	R	R	R	R	I	R	Yes
Ab35	R	R	R	R	R	R	R	R	R	R	R	S	S	Yes
Ab36	R	R	R	R	R	R	R	R	R	R	R	S	I	Yes
Ab37	R	R	R	R	R	R	R	R	R	R	R	S	S	Yes
Ab38	R	R	R	R	R	R	R	R	R	R	R	S	R	Yes
Ab39	R	R	R	R	R	R	R	R	R	R	R	S	R	Yes
Ab40	R	R	R	R	R	R	R	R	R	R	R	I	R	Yes
Ab41	R	R	R	R	R	R	R	R	R	I	R	R	R	Yes
Ab42	R	R	R	R	R	R	R	R	R	I	R	R	R	Yes
Ab43	R	R	R	R	R	R	R	R	R	R	R	S	S	Yes
Ab44	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab45	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab46	R	R	R	R	S	S	S	S	S	R	S	S	No	
Ab47	R	R	R	R	R	R	R	R	R	R	R	S	S	Yes
Ab48	R	R	R	R	R	R	R	R	R	R	R	R	S	Yes
Ab49	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab50	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab51	R	R	R	R	R	R	R	R	R	R	R	S	S	Yes
Ab52	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab53	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab54	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes

	R	R	R	R	R	R	R	R	R	R	R	S	S	Yes
Ab55	R	R	R	R	R	R	R	R	R	R	R	S	S	Yes
Ab56	R	R	R	R	R	R	R	R	R	R	R	S	S	Yes
Ab57	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab58	R	S	S	S	S	S	S	S	S	S	S	S	S	No
Ab59	R	R	R	R	R	R	R	R	R	R	R	R	S	Yes
Ab60	R	R	R	R	R	R	R	R	R	R	R	R	S	Yes
Ab61	R	R	R	R	R	R	R	R	R	R	S	R	S	Yes
Ab62	R	R	R	R	R	R	R	R	R	R	S	R	S	Yes
Ab63	R	R	R	R	R	R	R	R	R	R	S	R	S	Yes
Ab64	R	R	R	R	R	R	R	R	R	R	S	R	S	Yes
Ab65	R	R	R	R	R	R	R	R	R	R	S	R	S	Yes
Ab66	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab67	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab68	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab69	R	R	R	R	R	R	R	R	R	R	S	R	S	Yes
Ab70	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab71	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab72	R	R	R	R	R	R	R	R	R	R	S	R	S	Yes
Ab73	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab74	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab75	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab76	R	R	R	R	R	R	R	R	R	R	S	R	S	Yes
Ab77	R	R	R	R	R	R	R	R	R	R	S	R	S	Yes
Ab78	R	R	R	R	R	R	R	R	R	R	R	R	R	Yes
Ab79	R	R	R	R	R	R	R	R	R	R	S	R	S	Yes
Ab80	R	R	R	R	R	R	R	R	S	R	S	R	S	Yes
ATCC 19606	R	R	R	R	R	S	S	S	S	R	S	S	S	No
ATCC 17987	S	S	S	S	S	S	S	S	S	R	S	S	S	No
Total number of resistant isolates (%)	80 (100)	79 (99)	79 (99)	79 (99)	78 (97.5)	78 (97.5)	78 (97.5)	78 (97.5)	76 (95)	74 (92.5)	67 (83.8)	44 (55)	39 (48.8)	78 (97.5)

CAZ: ceftazidime, FEP: cefepime, CTX: cefotaxime, TZP: tazobactam/piperacillin, SAM: sulbactam /ampicillin, IPM: imipenem, CIP: ciprofloxacin, LEV: levofloxacin, AK: amikacin, CN: gentamicin, SXT: sulfamethoxazole/trimethoprim, DO: doxycycline, MIN: minocycline, XDR: extensively drug-resistant, R: resistant, I: intermediate, and S: sensitive.

Supplementary Table S3. Molecular characterization of resistance mechanisms of *A. baumannii* isolates and standard strains *A. baumannii* ATCC 19606 and 17978 to fluoroquinolones.

Isolate code	Fluoroquinolone-resistance determining regions				Plasmid-mediated quinolone resistance genes (<i>qnrA</i> , <i>qnrB</i> , <i>qnrC</i> , <i>qnrD</i> , <i>qepA</i> , and <i>oqxAB</i> genes were absent in all isolates)		
	<i>gyrA</i> gene		<i>parC</i> gene		<i>aac(6')-Ib</i> gene	<i>qnrS</i> gene	<i>qnrS+aac(6')-Ib</i> genes
	Detection	S83L Mutation	Detection	S80L Mutation			
Ab1	+	+	+	+	+	-	-
Ab2	+	+	+	+	+	-	-
Ab3	+	+	+	+	+	-	-
Ab4	+	+	+	+	+	-	-
Ab5	+	+	-	-	+	-	-
Ab6	+	+	+	+	-	-	-
Ab7	+	+	+	+	+	-	-
Ab8	-	-	+	+	+	-	-
Ab9	+	+	+	+	+	-	-
Ab10	+	+	+	+	-	-	-
Ab11	-	-	+	+	+	-	-
Ab12	+	+	+	+	+	-	-
Ab13	+	+	+	+	+	-	-
Ab14	+	+	+	+	+	-	-
Ab15	+	+	+	+	+	-	-
Ab16	+	+	+	+	-	-	-
Ab17	+	+	+	+	-	-	-
Ab18	+	+	+	+	+	-	-
Ab19	+	+	+	+	+	-	-
Ab20	+	+	+	+	+	-	-
Ab21	-	-	+	+	-	-	-
Ab22	+	+	+	+	+	-	-
Ab23	+	+	+	+	+	-	-
Ab24	+	+	+	+	-	-	-
Ab25	+	+	+	+	+	-	-
Ab26	+	+	+	+	+	-	-
Ab27	+	+	+	+	+	-	-
Ab28	+	+	+	+	+	-	-
Ab29	+	+	+	+	+	-	-
Ab30	+	+	+	+	+	-	-
Ab31	+	+	+	+	+	-	-
Ab32	+	+	+	+	+	-	-
Ab33	+	+	+	+	+	-	-
Ab34	+	+	-	-	+	-	-
Ab35	+	+	-	-	+	-	-
Ab36	+	+	+	+	-	-	-
Ab37	-	-	+	+	-	-	-
Ab38	-	-	+	+	+	-	-
Ab39	+	+	+	+	+	-	-
Ab40	+	+	+	+	+	-	-
Ab41	+	+	+	+	+	-	-
Ab42	+	+	+	+	+	-	-
Ab43	+	+	+	+	+	-	-
Ab44	+	+	+	+	+	-	-
Ab45	+	+	+	+	+	-	-
Ab46	+	+	+	+	+	-	-
Ab47	+	+	+	+	+	-	-

Ab48	+	+	+	+	+	-	-
Ab49	+	+	+	+	+	-	-
Ab50	+	+	+	+	+	-	-
Ab51	+	+	+	+	+	-	-
Ab52	+	+	+	+	+	-	-
Ab53	+	+	+	+	+	-	-
Ab54	+	+	+	+	+	-	-
Ab55	+	+	+	+	+	-	-
Ab56	+	+	+	+	-	-	-
Ab57	+	+	-	-	+	-	-
Ab58	-	-	+	+	+	-	-
Ab59	-	-	-	-	-	-	-
Ab60	+	+	+	+	+	+	+
Ab61	+	+	+	+	+	-	-
Ab62	+	+	+	+	+	-	-
Ab63	+	+	+	+	+	-	-
Ab64	-	-	+	+	+	-	-
Ab65	+	+	+	+	+	+	+
Ab66	+	+	+	+	+	-	-
Ab67	-	-	+	+	-	-	-
Ab68	+	+	+	+	+	-	-
Ab69	+	+	+	+	+	-	-
Ab70	+	+	+	+	+	-	-
Ab71	+	+	+	+	-	-	-
Ab72	+	+	+	+	-	-	-
Ab73	+	+	+	+	+	-	-
Ab74	+	+	+	+	+	-	-
Ab75	-	-	+	+	-	-	-
Ab76	-	-	+	+	-	-	-
Ab77	+	+	+	+	-	-	-
Ab78	+	+	+	+	-	-	-
Ab79	+	+	+	+	-	-	-
Ab80	+	+	+	+	-	-	-
ATCC 19606	+	-	+	-	-	-	-
ATCC 17987	+	-	+	-	-	-	-
Total number of positive isolates (%)	69 (86.3)	69 (86.3)	75 (93.8)	75 (93.8)	61 (76.3)	2 (2.5)	2 (2.5)

(+): Detected or present, (-): Not detected or absent, S80L: Ser80 to Leu and S83L: Ser83 to Leu.

Supplementary Table S4. Primers used in this study.

Gene or DNA region	Primer	Nucleotide Sequence (5' - 3')	Annealing temp (°C)	Amplicon size (bp)	Reference	
<i>recA</i> gene of <i>Acinetobacter</i> genus	<i>p-rA1</i>	CCTGAATCTTCTGGTAAAC	50	425	1	
	<i>p-rA2</i>	GTTTCTGGGCTGCCAACATTAC				
The intergenic spacer region region of <i>A. baumannii</i> (16S-23SrRNA ITS)	<i>p-Ab-ITSF</i>	CATTATCACGGTAATTAGTG	50	208	2	
	<i>p-Ab-ITSB</i>	AGAGCACTGTGCACTTAAG				
FQRDRs	<i>gyrA</i>	<i>gyrA-F</i>	56	343	3	
		<i>gyrA-R</i>				
	<i>parC</i>	<i>parC-F</i>	54	327	4	
		<i>parC-R</i>				
PMQR genes	<i>qnrA</i>	<i>qnrA-F</i>	57	579	5	
		<i>qnrA-R</i>				
	<i>qnrB</i>	<i>qnrB-F</i>	56	263		
		<i>qnrB-R</i>				
	<i>qnrS</i>	<i>qnrS-F</i>	53	427		
		<i>qnrS-R</i>				
	<i>qnrC</i>	<i>qnrC-F</i>	51	308	6	
		<i>qnrC-R</i>				
	<i>qnrD</i>	<i>qnrD-F</i>	50	645	7	
		<i>qnrD-R</i>				
Efflux pump genes	<i>aac(6')-Ib</i>	<i>aac(6')-Ib-F</i>	57	480	8	
		<i>aac(6')-Ib-R</i>				
	<i>oqxAB</i>	<i>oqxAB-F</i>	56	448	7	
		<i>oqxAB-R</i>				
	<i>qepA</i>	<i>qepA-F</i>	57	380	8	
		<i>qepA-R</i>				

Temp: temperature, F: forward, R: reverse, bp: base pair, FQRDRs: fluoroquinolone resistance-determining regions, and PMQR: plasmid-mediated quinolone resistance.

Supplementary Table S5. Effect of CCCP on the MICs of plain PCL nanoparticles, CIP, and LEV against representative fluoroquinolone (FQ)-resistant and extensively drug-resistant (XDR) *A. baumannii* isolates and standard strains *A. baumannii* ATCC 19606 and 17978.

Isolate code	MIC* ($\mu\text{g/ml}$)				MDF
	Plain	CIP	CIP + CCCP	Plain + CIP + CCCP	
Ab29	-	32	32	32	1
Ab30	-	32	8	8	4
Ab36	-	32	32	32	1
Ab60	-	128	16	16	8
Ab65	-	64	64	64	1
Ab71	-	64	64	64	1
Ab72	-	64	16	16	4
Ab77	-	64	64	64	1
ATCC 19606	-	0.5	0.5	0.5	1
ATCC 17987	-	0.25	0.25	0.25	1
Isolate code	MIC* ($\mu\text{g/ml}$)				MDF
	Plain	LEV	LEV + CCCP	Plain + LEV + CCCP	
Ab29	-	8	8	8	1
Ab30	-	8	2	2	4
Ab36	-	8	8	8	1
Ab60	-	16	4	4	4
Ab65	-	16	16	16	1
Ab71	-	8	8	8	1
Ab72	-	8	2	2	4
Ab77	-	8	8	8	1
ATCC 19606	-	0.5	0.5	0.5	1
ATCC 17987	-	0.25	0.25	0.25	1

MIC: minimum inhibitory concentration, CIP: ciprofloxacin, LEV: levofloxacin, PCL: poly ϵ -caprolactone, Plain: plain PCL nanoparticles, MDF: MIC decrease factor, and CCCP: carbonyl-cyanide-m-chlorophenylhydrazone.

*: The mean MIC value was reported after experiments were carried out in duplicate.

Supplementary Table S6. Distribution of *A. baumannii* isolates and standard strains *A. baumannii* ATCC 19606 and 17978 according to biofilm formation.

Isolate code	Biofilm formation	OD*	Isolate code	Biofilm formation	OD*
Ab1	N	0.022	Ab41	S	0.402
Ab2	W	0.095	Ab42	S	0.322
Ab3	S	0.522	Ab43	W	0.128
Ab4	S	0.489	Ab44	M	0.27
Ab5	S	0.451	Ab45	S	0.341
Ab6	S	0.422	Ab46	W	0.121
Ab7	W	0.115	Ab47	S	0.364
Ab8	S	0.391	Ab48	S	0.373
Ab9	N	0.028	Ab49	S	0.391
Ab10	S	0.371	Ab50	S	0.322
Ab11	W	0.132	Ab51	W	0.123
Ab12	S	0.42	Ab52	S	0.331
Ab13	S	0.373	Ab53	S	0.322
Ab14	W	0.123	Ab54	S	0.385
Ab15	S	0.521	Ab55	S	0.391
Ab16	S	0.432	Ab56	S	0.372
Ab17	S	0.367	Ab57	S	0.356
Ab18	S	0.395	Ab58	M	0.19
Ab19	S	0.421	Ab59	M	0.272
Ab20	S	0.478	Ab60	S	0.45
Ab21	S	0.341	Ab61	M	0.202
Ab22	S	0.357	Ab62	M	0.192
Ab23	S	0.364	Ab63	M	0.183
Ab24	W	0.131	Ab64	M	0.173
Ab25	S	0.398	Ab65	S	0.319
Ab26	S	0.38	Ab66	M	0.265
Ab27	S	0.351	Ab67	M	0.269
Ab28	S	0.284	Ab68	W	0.082
Ab29	S	0.371	Ab69	S	0.402
Ab30	S	0.541	Ab70	M	0.27
Ab31	S	0.356	Ab71	S	0.396
Ab32	S	0.381	Ab72	S	0.47
Ab33	S	0.394	Ab73	M	0.269
Ab34	S	0.322	Ab74	M	0.232
Ab35	S	0.341	Ab75	M	0.217
Ab36	S	0.542	Ab76	M	0.267
Ab37	M	0.25	Ab77	S	0.328
Ab38	S	0.361	Ab78	M	0.247
Ab39	W	0.125	Ab79	W	0.123
Ab40	S	0.375	Ab80	M	0.212
ATCC 19606	S	0.301	ATCC 17987	S	0.335

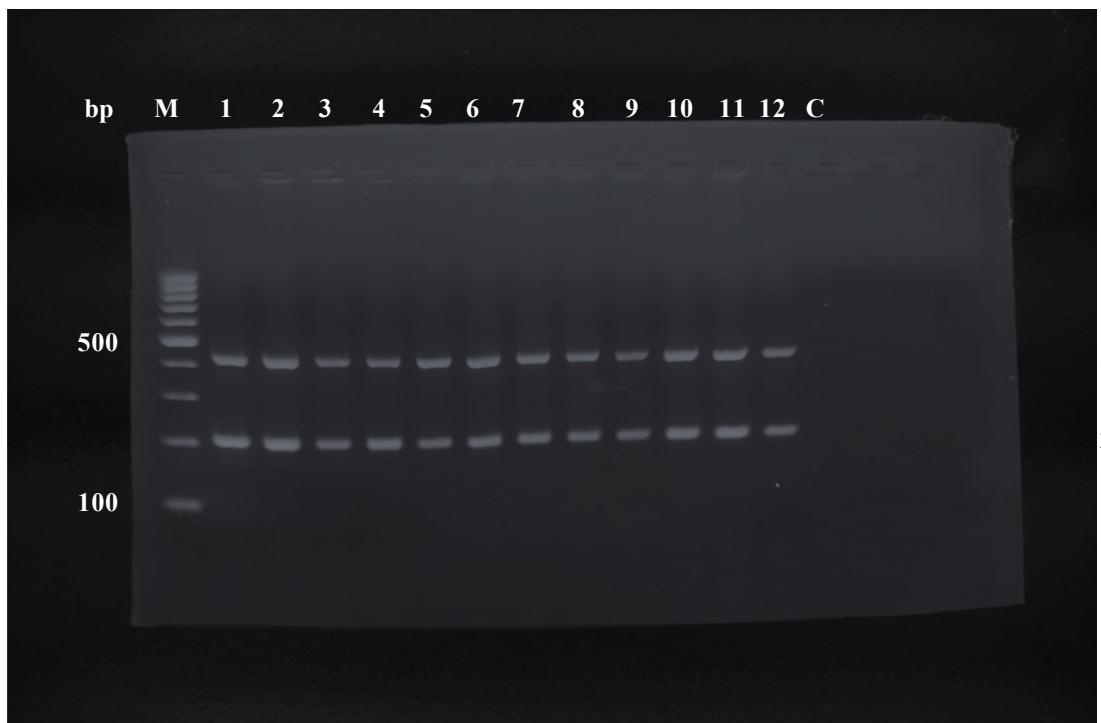
Total Number (%) of strong (S), moderate (M), week (W), and non (N)- biofilm forming isolates are 50 (62.5%), 17 (21.3%), 11 (13.8%) and 2 (2.5%) isolates, respectively.

*: The values represent the mean ± SD of each isolate's optical density (OD) of four wells.

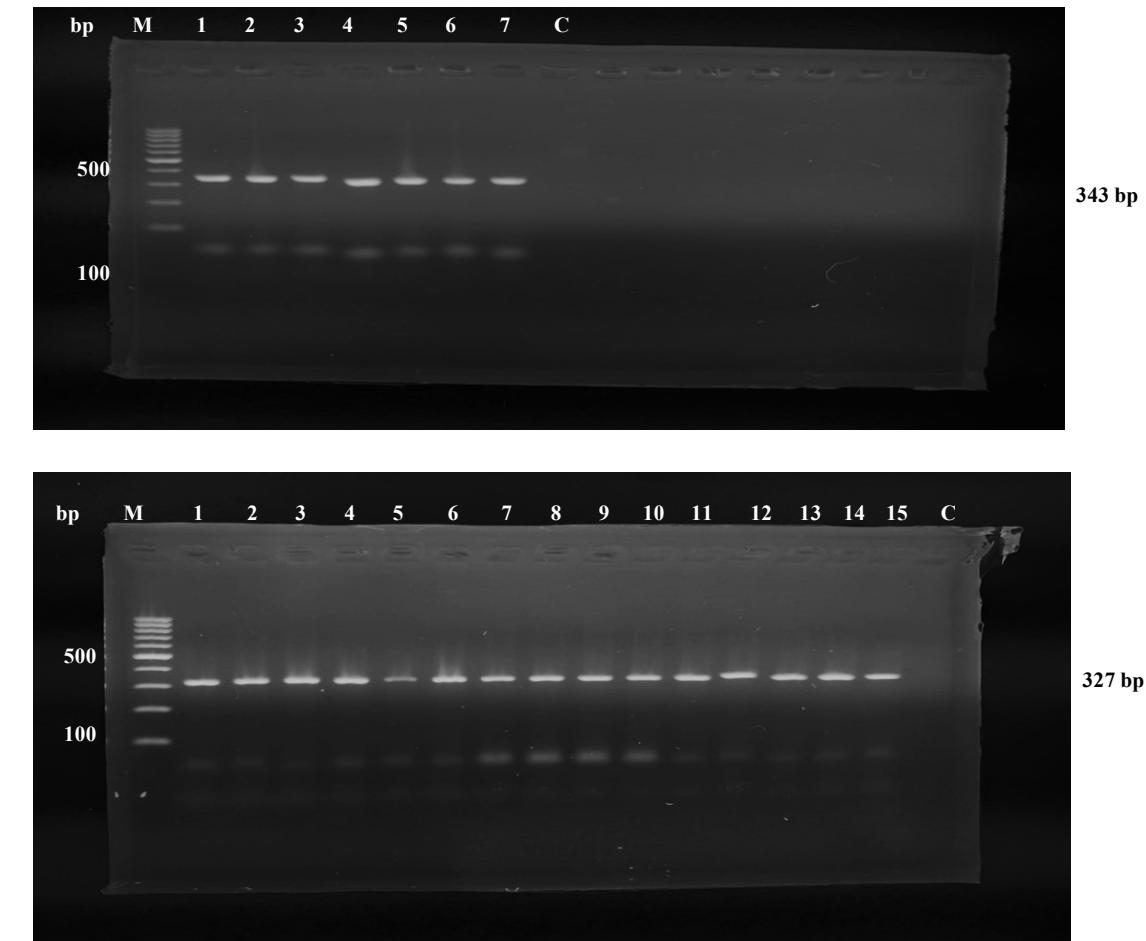
Supplementary Table S7. Ingredients of the prepared nanoparticles.

Parameter		Formula		F _{CIP}	F _{LEV}	Plain NPs
Ingredients % (w/v)	O	PCL		1.5	1.5	1.5
	W ₁	CIP		2	---	---
		LEV		---	2	---
	W ₂	PVA		0.5	0.5	0.5

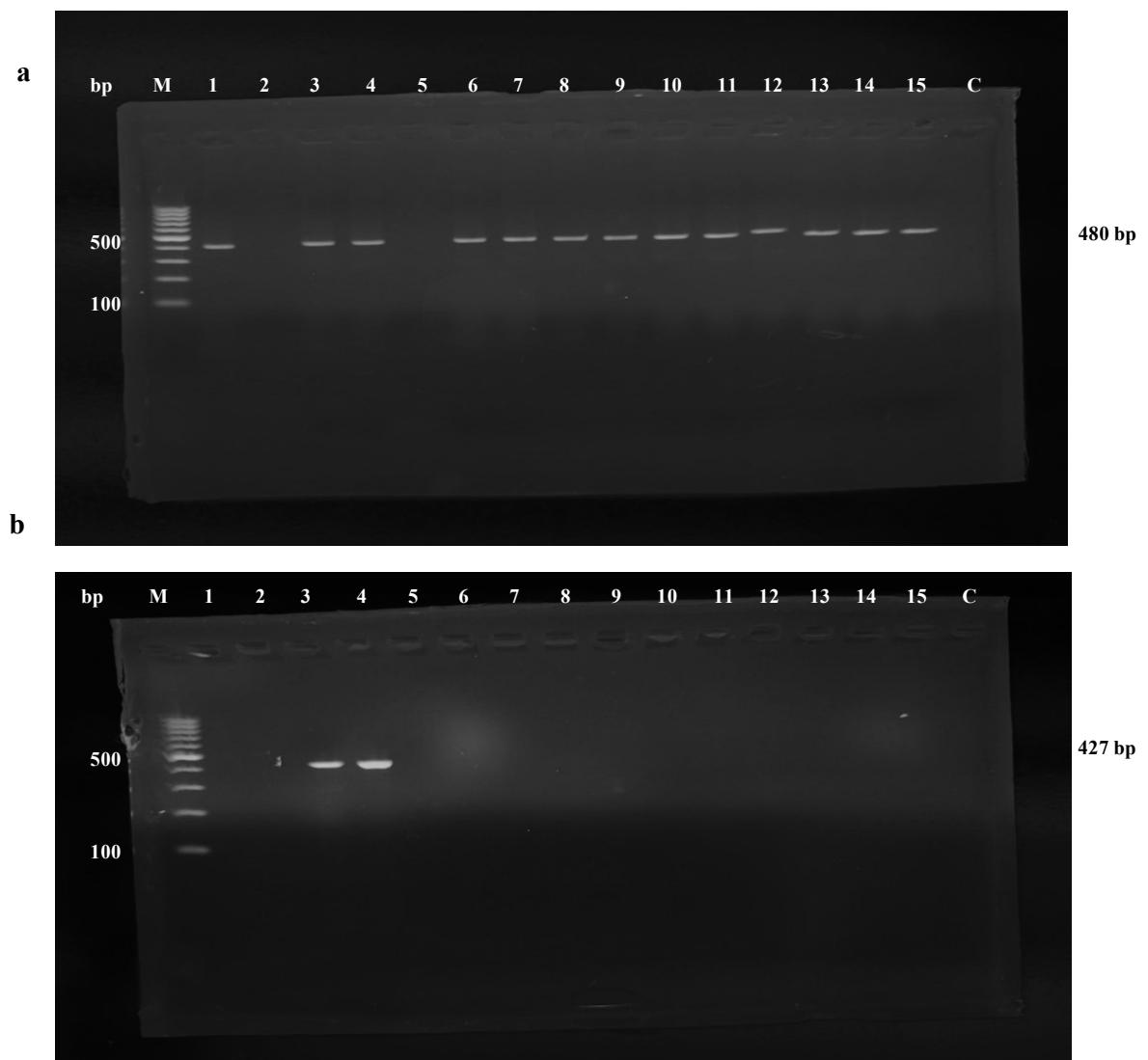
CIP: ciprofloxacin, LEV: levofloxacin, F_{CIP}: CIP-loaded nanoparticles, F_{LEV}: LEV-loaded nanoparticles, Plain NPs: plain PCL nanoparticles, O: organic phase, W₁: internal aqueous phase, W₂: external aqueous phase, PCL: poly ε-caprolactone, and PVA: polyvinyl alcohol.



Supplementary Fig. S1. Multiplex PCR for detection of *recA* gene (characteristic of the *Acinetobacter* genus) (425 bp) and *16S-23S rRNA* gene intergenic spacer (*ITS*) region (specific for *A. baumannii* spp.) (208 bp) in representative isolates of *A. baumannii*. Lane M: 100 bp DNA ladder, Lanes 1-10: positive isolates of *A. baumannii* (Ab1-10), Lanes 11 and 12 represent *A. baumannii* ATCC 19606 and 17978, respectively, Lane C: negative control (PCR reaction without DNA template), and bp: base pairs.



Supplementary Fig. S2. Detection of mutation in the fluoroquinolone resistance-determining regions (FQRDRs) after *HinfI* digestion of the PCR products of *gyrA* and *parC* genes. **a)** Mutation in the FQRDRs of *gyrA* is indicated by undigested fragment (343 bp) and Lanes 1-15 represent *A. baumannii* (Ab20-34) and Lanes 1-7 represent undigested fragments of *A. baumannii* (Ab1-7). **b)** Mutation in the FQRDRs of *parC* is indicated by undigested fragment (327 bp) and Lanes 1-15 represent undigested fragments of *A. baumannii* (Ab1-15). Lane M: 100 bp DNA ladder, Lane C: negative control (PCR reaction without DNA template) and bp: base pairs.



Supplementary Fig. S3. Detection of the plasmid-mediated quinolone resistance (PMQR) genes of PCR products of *aac(6')-Ib* and *qnrS* genes. **a)** *aac(6')-Ib* gene (480 bp) and Lanes 1-15 represent *A. baumannii* (Ab20-34), **b)** *qnrS* gene (427 bp) and Lanes 1-15 represent *A. baumannii* (Ab58-60 and Ab65-76). Lane M: 100 bp DNA ladder, Lane C: negative control (PCR reaction without DNA template) and bp: base pairs.

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