Adaptation of *Pseudomonas aeruginosa* to constant sub-inhibitory concentrations of quaternary ammonium compounds

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Electronic Supplementary Information

MIC determination – concentration used

In order to define the initial MIC (i.e the MIC of the bacterial strain prior to any exposure), two different sets of concentrations were used. The first set of concentrations used was from 0.06 to 32 mg/l and from 1 to 512 mg/l. Once the MIC for the selected was determined, the concentration interval was refined to have a more precise data.

Table S1

CTMA – concentration interval [c_{min} – c_{max}] mg/l	MIC [mg/l]	BAC – concentration interval [c_{min} – c_{max}] mg/l	MIC [mg/l]
[0.06 – 32]	>32	[0.06 – 32]	>32
[1 – 512]	128	[1 – 512]	128
[10 – 150]	110	[50 – 150]	80

Deconex 53 PLUS– concentration interval [c_{min} – c_{max}] mg/l	MIC [mg/l] (active substance)	Incidin® PLUS – concentration interval [c_{min} – c_{max}] mg/l	MIC [mg/l]
[0.06 – 32]	16	[0.06 – 32]	>32
[1 – 512]	16	[1 – 512]	64
[10 – 150]	15	[10 – 100]	40

Table S2. Average and standard deviations (4 data points) for the MIC before the exposure (cycle 0) during the exposure (cycles 1 to 10) to the QACs and after the stability period (QAC removed from the media for cycles 11 to 15; cycle 15)

QAC	Cycle (48 h)	Replicate	MIC [mg l^{-1}]		Replicate	MIC [mg l^{-1}]		Replicate	MIC [mg l^{-1}]		Replicate	MIC [mg l^{-1}]	
			Average	Standard deviation		Average	Standard deviation		Average	Standard deviation		Average	Standard deviation
CTMA	0	1	110.0	0.0	2	110.0	0.0	3	110.0	0.0	Control	110.0	0.0
CTMA	1	1	110.0	0.0	2	110.0	0.0	3	120.0	17.3	Control	111.4	9.0
CTMA	2	1	133.3	11.5	2	120.0	0.0	3	130.0	17.3	Control	112.0	4.5
CTMA	4	1	130.0	0.0	2	130.0	0.0	3	126.7	15.3	Control	113.3	5.2
CTMA	5	1	150.0	0.0	2	133.3	11.5	3	123.3	5.8	Control	103.3	11.2
CTMA	6	1	143.3	15.3	2	120.0	0.0	3	126.7	5.8	Control	111.7	4.1
CTMA	7	1	146.7	11.5	2	126.7	11.5	3	130.0	0.0	Control	114.3	7.9
CTMA	10	1	160.0	0.0	2	140.0	0.0	3	160.0	0.0	Control	116.4	5.0
CTMA	15	1	127.5	9.6	2	120.0	0.0	3	120.0	0.0	Control	108.3	7.5
BAC	0	1	80.0	0.0	2	80.0	0.0	3	80.0	0.0	Control	80.0	0.0
BAC	1	1	120.0	0.0	2	80.0	0.0	3	80.0	0.0	Control	80.0	0.0
BAC	2	1	127.5	5.0	2	120.0	0.0	3	120.0	0.0	Control	80.0	0.0
BAC	3	1	127.5	5.0	2	125.0	5.8	3	117.5	5.0	Control	80.0	0.0
BAC	4	1	130.0	0.0	2	130.0	0.0	3	130.0	0.0	Control	77.5	5.0
BAC	5	1	125.0	0.0	2	125.0	0.0	3	90.0	0.0	Control	70.0	0.0
BAC	6	1	125.0	0.0	2	125.0	0.0	3	100.0	0.0	Control	70.0	0.0
BAC	7	1	156.3	12.5	2	125.0	0.0	3	90.0	0.0	Control	70.0	0.0
BAC	8	1	150.0	0.0	2	131.3	12.5	3	100.0	0.0	Control	70.0	0.0
BAC	9	1	150.0	0.0	2	112.5	14.4	3	106.3	12.5	Control	80.0	0.0
BAC	10	1	150.0	0.0	2	150.0	0.0	3	95.0	5.8	Control	72.5	5.0
BAC	15	1	150.0	0.0	2	150.0	0.0	3	90.0	0.0	Control	70.0	0.0

Table S3: Cross-resistance results for sequential exposures of P. aeruginosa to BAC followed by CTMA or CTMA followed by BAC. Populations exposed to CTMA were tested against BAC and exposed to BAC were tested against CTMA to investigate cross-resistance. This table summarizes the MIC obtained for the cross-resistance experiments.

Exposed to CTMA			**Exposed to BAC**			**Exposed to Deconex® 53 PLUS**		
Cycle (48 h)	MIC	Cycle (48 h)	MIC	MIC [mg l^{-1}]	MIC [mg l^{-1}]	Cycle (48 h)	MIC	MIC [mg l^{-1}]
0	CTMA[a]	0	CTMA[a]	110	110	0	CTMA[a]	200
	BAC[a]		BAC[a]	80	80		BAC[a]	80
5	CTMA	5	CTMA	150[b] / 200[c]	130[b] / 150[c]	5	CTMA	NA
	BAC		BAC	80[b] / 150[c]	90[b] / 125[c]		BAC	NA
10	CTMA	10	CTMA	150[b] / 200[c]	140[b] / 160[c]	10	CTMA	200[c] / 250[b]
	BAC		BAC	90[b] / 150[c]	90[b] / 140[c]		BAC	100[c] / 140[b]
15[d]	CTMA	15[d]	CTMA	150	90[b] / 150[c]	15[d]	CTMA	200[c]/250[b]
	BAC		BAC	80	150		BAC	100[c]/160[b]

[a] control without QAC, [b] replicate 1; [c] replicates 2 and 3; [d] MIC obtained after the stability period

Table S4. Average and standard deviation data of the Inhibition Zone Diameters (IZD) obtained with the disc diffusion method.

cycle	Treatment	Amikacin IZD [mm]		Ceftazidime IZD [mm]		Ciprofloxacin IZD [mm]		Gentamycin IZD [mm]		Cefepime IZD [mm]		Imipenem IZD [mm]	
		Average	Std deviation	Average	Std deviation	Average	Std deviation	Average	Std deviation	Average	Std deviation	Average	Std deviation
0	**NA**	24	1	25	0.9	35.1	0.8	21	2.2	31.2	1.2	24.1	1.1
5	**CTMA**	26.6	0.9	29.8	1.2	36.9	1.5	20.7	1.1	35.6	1.4	27.7	0.7
10	**CTMA**	22.8	2	26.6	1.1	34.8	1	18.7	2	32.6	1	27.2	0.8
15	**CTMA**	24	1.7	25.8	0.8	33.8	1.2	20	0.9	30.2	1.8	24.8	1.5
5	**BAC**	28.9	2.3	30	2.5	38.1	2	24.4	1.2	35.4	1.8	27.8	2.6
10	**BAC**	26.6	1.2	30.7	1.9	37	1	22.8	0.7	33.2	1.3	28	1.6
15	**BAC**	27.2	0.7	27.3	1.2	30.8	1.5	23.6	1.2	28.8	2.4	26.6	1.6
5	**Control**	27.1	2.6	27.9	5.7	33.7	3	19.9	1.6	33.3	3.6	26.1	1
10	**Control**	25.4	2.4	25.8	1.4	31.7	1.6	21.1	2.1	27.1	1.4	25.2	0.8

cycle	Treatment	Levofloxacin IZD [mm]		Meropenem IZD [mm]		Minocycline IZD [mm]		Tobramycin IZD [mm]		Piperacillin-tazobactam IZD [mm]	
		Average	Std deviation	Average	Std deviation	Average	Std deviation	Average	Std deviation	Average	Std deviation
0	**NA**	26.7	1.1	36.9	1.5	18.4	1.4	25.6	1.1	30.2	1.1
5	**CTMA**	28.6	1.7	38.1	1.9	20.4	1.7	27.6	1.5	31.2	1.2
10	**CTMA**	27.7	1	35	1	13.2	1.6	26.6	1	27.9	2
15	**CTMA**	26	0.7	35.6	0.9	15.4	1.1	22.6	1.3	28	1.3
5	**BAC**	32	1.1	35.6	2	14	1.3	24.3	0.7	28.9	1.5
10	**BAC**	30.7	0.9	35.5	1.2	15.8	0.8	24.3	0.8	27.8	1
15	**BAC**	27	1.8	34	1.1	11.2	2	23.8	2.3	26.6	1.2
5	**Control**	28	2.3	35.8	4.4	10	2.1	24.3	0.9	30.4	2.7
10	**Control**	27	1.1	33.3	1	14.7	0.7	23.2	1	26.4	0.9