



- 1 **Figure S1** Structural analysis of lipid A from *P. aeruginosa*. (A) The structure of WT
- 2 lipid A (*m/z* 1445) represents a bisphosphorylated, penta-acylated lipid A molecule on
- 3 the fatty acyl chain. (B) Different lipid A component modifications, including
- 4 dephosphorylation (-PO<sub>3</sub>), deacylation (-3-OH C10), acylation (+3-OH C10),
- 5 palmitoylation (+C16), dehydroxylation (-OH), hydroxylation (+OH) and
- 6 glycosylation (+L-Ara4N).

7 **Table S1.** primers used in this study.

Gene	Sequence (5'→3')	Amplicon size (bp)	Reference
Primers for sequencing			
<i>pmrAB</i>	F: TCACTGAAACGAGGCTGCCATGAG R: CCAATGCGCAGGCTATCAGATATGT	2138	This study
<i>phoP</i>	F: CGCCATATGAAACTGCTGGTAGTGGAA R: CGCCTCGAGCCGGCAGCGCTCGGTGAA	690	This study
<i>phoQ</i>	F: CGGCATATGATCCGTTCCCTGCGCATC R: CGGCTCGAGGACTGTAGCGAAACGTATG	1359	This study
<i>oprH</i>	F: CGGCATATGAAAGCACTCAAGACTCTC R: CGGCTCGAGGAACTTGTAGTTGGCGCC	615	This study
<i>parR</i>	F: GCATATAATGCCAGCCGATT R: ACCACCAGCAGGTTCTTGTC	1055	(1)
<i>parS</i>	F: CTATTCGCTGGTGGAAAAGC R: GTTAAGCCTCCGCTGTCAAC	1162	(1)
<i>cprR</i>	F: CGCAGTATCCGAAGGAAGAA R: CCCTTCCTCTTCCATCATCA	1154	(1)
<i>cprS-1</i>	F: TCTGATCCATAACCCTGCACA R: CTGTTCCCTCGAGCAGTTCCT	1002	(1)
<i>cprS-2</i>	F: ACCTGGATCGTCACCCTGT R: TCTTCTCGGCGATCAAGTTC	1044	(1)
<i>colR</i>	F: GCGAAGGAGTGGGACATGCGA R: CGAGGCTCTGCTTATACTCC	710	This study
<i>colS</i>	F: CTTGCTGCATACGGTGCATG R: TGAATTTTTTCGTCAAGCAAC	1307	This study
Primers for qRT-PCR			
<i>rspL</i>	F: GTGGTGAAGGTCACAACCTG R: CCTGCTTACGGTCTTTGACA	135	(2)
<i>pmrA</i>	F: CACCAGGTGACCCTGTCC R: CGTAGAGGCTCTGCTCCAGT	124	(2)
<i>phoP</i>	F: TCTACCGGGTCAGCGAATAC R: GATCAGGATCGGGAAGGACT	122	(2)
<i>pmrH</i>	F: GTTCGTCAGCGACGACAGT R: AAACCGGGCTCGATAACTTC	129	(2)
<i>parR</i>	F: AGAATGGTCTGCAGGTGTGC R: CGGCTTGATCACGTAGTCGT	134	(2)
<i>cprR</i>	F: CGCCTGGAAGATCCTTGAGT R: CACGTTGAGGGTGTTGCTTT	115	(2)

9 **Table S2.** Lipid A species detected for the colistin resistant strains (n=2) and hetero-resistant strains (n=9)

Strain	WT		WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
	-3OH C10	WT	WT	WT		WT	-PO3	WT	+4-OH	+3-OH	+2-OH	WT	+2-OH	+3-OH
	-PO3	-3OH C10	-PO3	-OH		+OH	+Ara4N	+Ara4N	C10	C10	C10	+C16	+Ara4N	+Ara4N
	m/z 1195	m/z 1274	m/z 1366	m/z 1429	m/z 1445	m/z 1461	m/z 1496	m/z 1576	m/z 1600	m/z 1616	m/z 1632	m/z 1684	m/z 1731	m/z 1747
PAO1	/	/	10.6±2.8	20.6±4.4	100±0.0	8.4±2.3	/	/	/	14.4±2.2	/	/	/	/
CST-FR														
TL1671	/	/	14.2±3.1	23.4±3.6	100±0.0	14.9±1.1	30±0.5	14.9±2.2	9.0±1.7	31.0±2.8	6.1±0.4	/	/	3.5±0.2
TL2204	/	/	18.3±7.8	42.5±1.5	100±0.0	10.0±0.7	5.4±1.7	/	4.0±0.8	14.3±2.2	/	/	/	/
CST-HR														
TL1722	SP	3.7±2.9	/	6.4±2.5	20.8±1.8	100±0.0	6.73±4.3	/	/	/	7.3±0.8	/	/	/
	RSP	/	/	5.0±0.1	10.5±1.5	100±0.0	10.4±1.2	8.5±1.6	15.9±3.7	11.5±2.4	8.3±1.8	/	5.1±0.8	8.0±0.5
TL1736	SP	/	/	21.3±1.1	49.7±4.4	100±0.0	10.2±0.4	/	/	2.8±0.1	15.8±0.2	1.6±0.0	2.2±0.1	/
	RSP	/	/	3.8±0.8	16.6±0.9	100±0.0	7.7±0.7	9.3±2.7	10.3±2.4	/	26.8±2.4	5.3±1.9	/	3.0±0.6
TL1744	SP	/	/	10.3±1.9	10.3±2.5	100±0.0	6.5±2.3	/	/	/	3.5±0.6	/	2.5±0.1	/
	RSP	/	/	8.9±1.6	27.4±3.1	100±0.0	7.9±0.8	/	/	/	6.4±1.4	/	/	5.4±1.2
TL2294	SP	/	/	/	15.5±1.9	100±0.0	9.3±1.1	/	/	8.6±3.2	7.2±0.3	/	/	/
	RSP	10.2±1.8	11.0±0.7	3.7±5.2	22.7±2.2	100±0.0	10.9±0.1	/	13.3±1.1	8.8±1.0	80.1±17.5	11.2±2.0	/	5.9±2.0
TL2314	SP	/	/	10.4±1.0	24.5±1.6	100±0.0	7.5±0.8	/	/	/	21.7±0.4	/	/	/
	RSP	/	/	/	7.0±0.2	100±0.0	9.5±0.2	3.9±0.9	10.6±0.3	1.9±1.7	25.9±0.9	3.1±0.7	/	2.6±0.6
TL2917	SP	11.7±0.2	6.5±0.3	8.5±1.0	36.0±0.4	100±0.0	11.8±0.5	/	/	/	10.8±0.9	/	/	/
	RSP	/	/	/	23.9±2.5	100±0.0	8.6±2.7	11.1±1.7	5.7±1.6	/	12.8±0.4	/	/	2.8±0.9

TL2967	SP	/	/	3.5±0.3	41.5±0.9	100±0.0	15.5±0.2	/	/	3.5±0.3	18.4±0.6	2.5±0.3	/	/	/
	RSP	/	/	/	8.0±4.2	100±0.0	19.7±1.9	/	20.2±3.0	/	17.6±1.0	/	/	/	/
TL3008	SP	/	4.9±1.1	14.6±0.8	27.1±0.8	100±0.0	15.6±0.4	/	/	5.4±0.5	32.4±3.6	22.4±23.7	/	/	/
	RSP	/	/	/	9.5±0.1	100±0.0	29.6±0.6	/	19.7±2.4	/	31.3±2.6	9.8±0.6	/	/	5.2±0.9
TL3086	SP	/	2.3±0.9	13.8±5.1	25.1±0.0	100±0.0	17.3±0.4	/	/	2.3±0.1	23.7±0.5	3.6±0.2	/	/	/
	RSP	/	/	/	10.2±1.2	100±0.0	30.6±1.7	/	14.0±1.4	/	29.8±2.6	11.8±2.0	/	/	6.2±1.0

10 Values represent mean ± SD of the relative peak intensities. Every strain was conducted in triplicate by MALDI-TOF MS;

11 CST-FR, colistin fully-resistant isolates; CST-HR, colistin hetero-resistant isolates; SP, susceptible populations; HSP, hetero-resistant

12 subpopulations.

13 <sup>a</sup> "/" represents ion relative intensity <1.0 or not detected.

14 **Table S3** Frequencies of mutations in two colistin-resistant and nine colistin-hetero-  
 15 resistant *P. aeruginosa*.

Isolates	Frequencies of mutation to rifampicin (300 µg/ml) resistance	Mutator phenotype <sup>a</sup>
PAO1	$1.44 \times 10^{-10}$	/
TL1671	$2.41 \times 10^{-10}$	-
TL2204	$1.05 \times 10^{-10}$	-
TL1722	$2.30 \times 10^{-10}$	-
<b>TL1736</b>	<b><math>1.52 \times 10^{-6}</math></b>	+
TL1744	$2.00 \times 10^{-9}$	-
TL2294	$7.20 \times 10^{-10}$	-
<b>TL2314</b>	<b><math>2.20 \times 10^{-6}</math></b>	+
<b>TL2917</b>	<b><math>7.78 \times 10^{-6}</math></b>	+
<b>TL2967</b>	<b><math>1.67 \times 10^{-8}</math></b>	+
<b>TL3008</b>	<b><math>9.10 \times 10^{-9}</math></b>	+
TL3086	$1.25 \times 10^{-9}$	-

16 <sup>a</sup> The strain was considered a mutator phenotype when the mutation frequency  
 17 conferring rifampicin resistance was at least 20-fold higher than that observed for the  
 18 wild-type strain PAO1.

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## 20 **References**

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