

## SUPPLEMENTAL MATERIAL

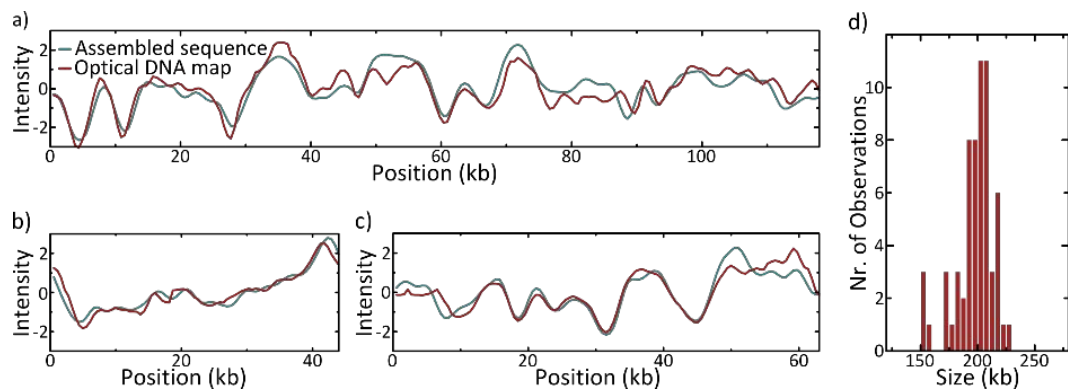
**Table S1** Isolation data

Species	Strain	Isolation site	Treatment <sup>†</sup>	Treated abroad <sup>†</sup>
<i>Pseudomonas aeruginosa</i>	CCUG 70744	Sputum	Meropenem	-
<i>Escherichia coli</i>	CCUG 70745	Rectum	-	-
<i>Klebsiella pneumoniae</i>	CCUG 70742	Urine	-	-
<i>Proteus mirabilis</i>	CCUG 70746	Rectum	-	Balkan
<i>Acinetobacter baumannii</i>	CCUG 70743	Wound	-	Middle east
<i>Klebsiella pneumoniae</i>	CCUG 70747	Wound	Cefotaxime	Balkan

<sup>†</sup> No information marked with dash (-).

**Table S2** Intrinsic resistance genes in the reference genomes excluded from further analysis

Strain	Gene	Function	Accession number
<i>P. aeruginosa</i> PAO1	<i>aph(3')-IIb</i>	Aminoglycoside phosphotransferase	X90856
	<i>bla<sub>OXA-50-like</sub></i>	β-lactamase	AY306130
	<i>bla<sub>PAO</sub></i>	β-lactamase	FJ666065
	<i>catB7</i>	Chloramphenicol acetyltransferase	AF036933
	<i>fosA</i>	Enzymatic fosfomycin resistance	NZ_ACWU01000146
<i>K. pneumoniae</i> MGH 78578	<i>bla<sub>SHV-11</sub></i>	β-lactamase	EF035558
	<i>fosA</i>	Enzymatic fosfomycin resistance	NZ_AFBO01000747
	<i>oqxAB</i>	Quinolone efflux pump	EU370913
<i>P. mirabilis</i> HI4320	<i>cat</i>	Chloramphenicol acetyltransferase	M11587
	<i>tet(I)</i>	Tetracycline efflux pump	ACLE01000065
<i>A. baumannii</i> ATCC 17978	<i>bla<sub>ADC-25</sub></i>	β-lactamase	EF016355
	<i>bla<sub>OXA-66</sub></i>	β-lactamase	FJ360530



**Fig. S1** Comparison between experimentally generated optical DNA maps (red) and theoretical optical DNA maps based on assembled sequences (teal) for non-novel plasmids **(a)** pKpn70747\_2 in *K. pneumoniae* CCUG 70747 (118 kb), **(b)** pEco70745\_2 in *E. coli* CCUG 70745 (44 kb), and **(c)** pKpn70742\_2 in *K. pneumoniae* CCUG 70742 (63 kb). Intensity (y-axis) refers to the normalized emission intensity along the DNA molecule (x-axis) which correlates coarsely with the underlying DNA sequence. **(d)** Histogram displaying the size distribution of individual plasmid molecules found in *P. mirabilis* CCUG 70746, demonstrating a high variability of the plasmid within the sample.

**Table S3** Chromosomal genes screened for mutations conferring resistance

Reference strain	Function	Gene	Locus tag
<i>P. aeruginosa</i> PAO1	Quinolone targets	<i>gyrA</i>	PA3168
		<i>gyrB</i>	PA0004
		<i>parC</i>	PA4964
		<i>parE</i>	PA4967
	β-lactamase	<i>ampC</i>	PA4110
	Penicillin-binding protein	<i>dacB</i>	PA3047
		<i>dacC</i>	PA3999
		<i>ftsI</i>	PA4418
		<i>mrcB</i>	PA4700
		<i>pbpA</i>	PA4003
		<i>pbpC</i>	PA2272
		<i>pbpG</i>	PA0869
	Drug efflux pumps	<i>ponA</i>	PA5045
		<i>mexA</i>	PA0425
		<i>mexB</i>	PA0426
		<i>mexC</i>	PA4599
		<i>mexD</i>	PA4598
		<i>mexE</i>	PA2493
		<i>mexF</i>	PA2494
		<i>mexX</i>	PA2019
		<i>mexY</i>	PA2018
		<i>oprJ</i>	PA4597
		<i>oprM</i>	PA0427
	Porin	<i>oprN</i>	PA2495
		<i>oprD</i>	PA0958
	Regulatory proteins	<i>ampD</i>	PA4522
		<i>ampR</i>	PA4109
<i>czcR</i>		PA2523	
<i>czcS</i>		PA2524	
<i>mexR</i>		PA0424	
<i>mexT</i>		PA2492	
<i>mexZ</i>		PA2020	
<i>nalC</i>		PA3721	
<i>nalD</i>		PA3574	
<i>nfxB</i>		PA4600	
<i>E. coli</i> str. K-12 substr. MG1655	Quinolone targets	<i>gyrA</i>	b2231
		<i>gyrB</i>	b3699
		<i>parC</i>	b3019
		<i>parE</i>	b3030
<i>K. pneumoniae</i> MGH 78578	Quinolone targets	<i>gyrA</i>	KPN_02640
		<i>gyrB</i>	KPN_03437
		<i>parC</i>	KPN_03444
		<i>parE</i>	KPN_04102
	Drug efflux pumps	<i>acrA</i>	KPN_00444
		<i>acrB</i>	KPN_00443
	Regulatory proteins	<i>acrR</i>	KPN_00445
		<i>marA</i>	KPN_01624
		<i>marR</i>	KPN_01625
		<i>ramA</i>	KPN_00556
		<i>ramR</i>	KPN_00555
		<i>soxR</i>	KPN_04463
		<i>soxS</i>	KPN_04462
	Outer membrane protein	<i>tolC</i>	KPN_03449
	<i>P. mirabilis</i> HI4320	Quinolone targets	<i>gyrA</i>
<i>gyrB</i>			HMPREF0693_0114
<i>parC</i>			HMPREF0693_2557
<i>parE</i>			HMPREF0693_2558
<i>A. baumannii</i> ATCC 17978	Quinolone targets	<i>gyrA</i>	A1S_2626
		<i>gyrB</i>	A1S_0004
		<i>parC</i>	A1S_0194
		<i>parE</i>	A1S_3359

**Table S4** MICs and EUCAST clinical resistance classifications

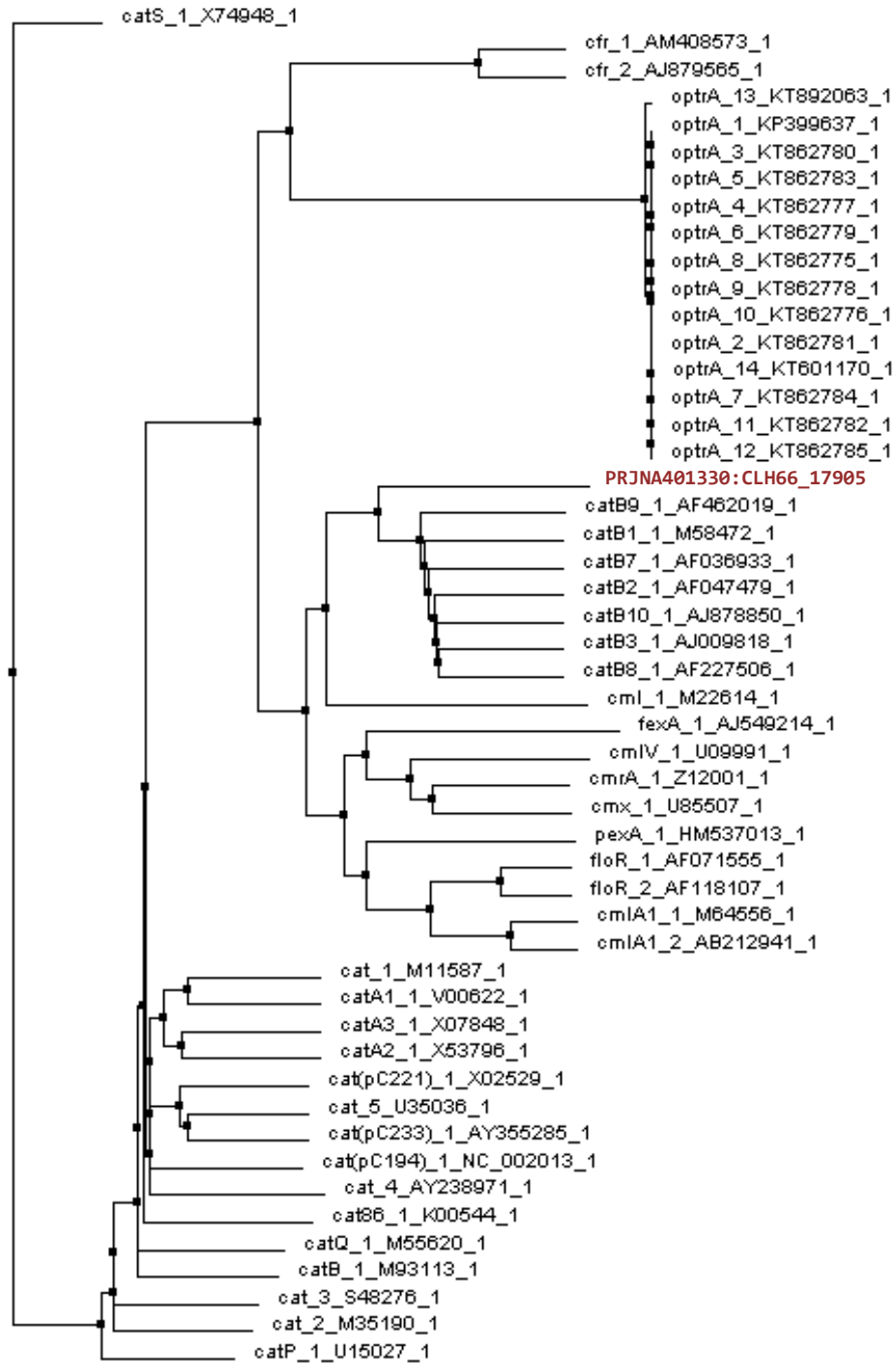
Antibiotic class	Antibiotic	<i>P. aeruginosa</i>		<i>E. coli</i>		<i>K. pneumoniae</i>		<i>P. mirabilis</i>		<i>A. baumannii</i>		<i>K. pneumoniae</i>	
		CCUG 70744*		CCUG 70745*		CCUG 70742*		CCUG 70746*		CCUG 70743*		CCUG 70747*	
Carbapenems	Ertapenem	>32	iR	>32	R	4	R	>32	R	>32	iR	>32	R
	Imipenem	>32	R	>32	R	0.5	S	>32	R	>32	R	>32	R
	Meropenem	>32	R	>32	R	0.5	S	32	R	>32	R	>32	R
Cephalosporins	Cefuroxime	>256	iR	>256	R	>256	R	>256	R	>256	iR	>256	R
	Cefotaxime	>32	iR	>32	R	>32	R	>32	R	>32	iR	>256	R
	Ceftazidime	256	R	>256	R	32	R	>256	R	>256	-	>256	R
	Ceftriaxone	>32	iR	>32	R	>32	R	>32	R	>32	iR	>32	R
Monobactams	Aztreonam	96	R	>256	R	64	R	2	I	>256	-	>256	R
Penicillins	Amoxicillin	>256	iR	>256	R	>256	R	>256	R	>256	iR	>256	R
	Ampicillin	>256	iR	>256	R	>256	iR	>256	R	>256	iR	>256	iR
	Amoxicillin/clavulanic acid	>256/2	iR	>256/2	R	>256/2	R	>256/2	R	>256/2	iR	>256/2	R
	Piperacillin/Tazobactam	>256/4	R	>256/4	R	256/4	R	32/4	R	>256/4	-	>256/4	R
Aminoglycosides	Amikacin	16	I	16	I	6	S	>256	R	64	R	32	R
	Gentamicin	4	S	1	S	2	S	>256	R	64	R	2	S
	Tobramycin	1	S	16	R	16	R	>256	R	64	R	16	R
Fluoroquinolones	Ciprofloxacin	1	I	>32	R	>32	R	>32	R	>32	R	>32	R
	Levofloxacin	8	R	>32	R	2	I	32	R	>32	R	32	R
	Moxifloxacin	8	R	>32	R	4	R	32	R	>32	-	>32	R
Sulfonamides	Trimethoprim/sulfamethoxazole	>32/608	iR	>32/608	R	>32/608	R	>32/608	R	>32/608	R	>32/608	R
Tetracyclines	Tetracycline	64	iR	>256	R	192	R	32	iR	>256	-	4	R
	Tigecycline	16	iR	1	S	4	R	32	R	8	-	1	S
Others	Colistin	0.5	S	0.25	S	2	S	>256	iR	1	S	2	S
	Fosfomycin	>256	-	0.25	S	32	S	8	S	1024	iR	64	R
	Chloramphenicol	>256	iR	64	R	4	S	256	R	>256	-	16	R

\*S = susceptible, I = intermediate, R = resistant, iR = intrinsically resistant. Units are mg l<sup>-1</sup>.

**Table S5.** Closest matching plasmids

Strain	Plasmid	Length (bp)	Closest match in GenBank	Length (bp)	Accession number	Isolation county	Alignment identity	Gaps	Same ARGs*
<i>E. coli</i> CCUG 70745	pEco70745_1	107 323	<i>Escherichia coli</i> isolate Co6114 plasmid pC06114_1	129 085	CP016035.1	Germany	67 %	19 %	No
	pEco70745_2	44 006	<i>Escherichia coli</i> strain OM26 plasmid pOM26-1	45 122	KP776609.1	Oman	98 %	3 %	Yes
	pEco70745_3	3 608	<i>Salmonella enterica</i> subsp. enterica serovar Typhimurium var. 5- str. CFSAN001921 plasmid unnamed	3 609	CP006052.1	USA	98 %	0 %	-
	pEco70745_4	1 934	<i>Escherichia coli</i> strain G3/10 plasmid pSYM3	1 934	KM107839.1	Germany	100 %	0 %	-
	pEco70745_5	1 780	<i>Escherichia coli</i> strain EC08 plasmid pEC08-1 RepA gene	1 980	JX238440.1	Sweden	93 %	0 %	-
	pEco70745_6	1 459	<i>Escherichia coli</i> strain ST648 plasmid pEC648_7	1 459	CP008721.1	China	100 %	0 %	-
<i>K. pneumoniae</i> CCUG 70742	pKpn70742_1	65 276	<i>Klebsiella pneumoniae</i> strain ATCC BAA-2146 plasmid pHg	85 161	CP006662.2	USA	67 %	26 %	No
	pKpn70742_2	63 075	<i>Enterobacter cloacae</i> strain INSRA17313-1 plasmid pUR17313-1	63 584	KP061858.1	Portugal	97 %	3 %	Yes
	pKpn70742_3	4 426	<i>Klebsiella pneumoniae</i> strain 0773 plasmid pKpn114, complete sequence	4 211	EU932690.1	China	96 %	7 %	-
	pKpn70742_4	1 780	<i>Escherichia coli</i> strain EC08 plasmid pEC08-1 RepA gene	1 780	JX238440.1	Sweden	93 %	0 %	-
<i>P. mirabilis</i> CCUG 707466	pPmi70746_1	191 769	<i>Salmonella enterica</i> subsp. enterica serovar Corvallis plasmid pRH-1238	187 683	KR091911.1	Germany	78 %	20 %	No
<i>A. baumannii</i> CCUG 70743	pAba70743_1	10 880	<i>Acinetobacter baumannii</i> plasmid pAB120	10 879	JX069966.1	Lithuania	100 %	0 %	Yes
<i>K. pneumoniae</i> CCUG 70747	pKpn70747_1	186 310	<i>Klebsiella pneumoniae</i> JM45 plasmid p1	317 154	CP006657.1	China	52 %	41 %	No
	pKpn70747_2	117 859	<i>Klebsiella pneumoniae</i> strain Kpn-1870 plasmid pGR-1870	116 047	KF874498.1	Greece	89 %	7 %	Yes
	pKpn70747_3	32 773	<i>Salmonella enterica</i> subsp. enterica serovar Typhi linear plasmid pBSSB1	27 037	AM419040.1	Indonesia	73 %	21 %	-

\* Antibiotic resistance genes (ARGs).



**Fig. S2 (a)** Multiple sequence alignment of all phenicol resistance proteins in the ResFinder database and the *cat*-like protein encoded by the chromosome of *E. coli* CCUG 70745. The putative phenicol resistance gene (protein id PRJNA401330:CLH66\_17905) is most similar to the group of CatB enzymes. The alignment was done using Clustal Omega and the figure depicts a neighbour joining tree using the BLOSUM62 distance matrix.

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> PRJNA401330:CLH66_17905
MKIMKINNIDVMKENDIVFSNTAGFWSGPVEIECPAEIRQGVYQVDKIGAF
SYMGGKSTHMLHIESIGRFCAIAGNVVAGAMEHPAHLSPHPIMQGVFKWK
ALDDFKLKNKMDLWKSRTFNNQINKDRFGKIKIGNDVWIGEGAFIRRGVKI
GDGAIASHSVSKDVAPYSIVGGVPAKHIFRFEDNIEQLIEIKWWDYG
LSALEGVDFTDINKAIKKIRENIDSGVAEKFTPNVIRINKDGVAEKI*

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**Fig. S2 (b)** The predicted protein sequence of the putative novel phenicol inactivating enzyme in *E. coli* CCUG 70745 .

AF462019_1	1	MNFFTSPFSGIPLDQVTPNPIIVGKHSYSGYYHGHSFDDCVRYLHPER	50
		... ..:  . ... .   . ... : ... ...: ...:....	
CLH66_17905	1	MKIM--KINNI----DVMKENDIV--FSNTAGFWSGPVEIECPAEIRQGV	42
AF462019_1	51	DDVDKL-----VIGSFCSIGSGAVFMMAGNQGHRSDWI	83
		..   : .  .  : ...    ... ...:	
CLH66_17905	43	<u>YQVDKIGAFSYMGGKSTHMLHIESIGRFCAIAGNVV--AGAMEHPAHL</u>	89
AF462019_1	84	STFP-----FFYQDNDNFA-DARDGFTRS-----GDTIIGHDV	115
		..   ...: : ... ...:   ... : :	
CLH66_17905	90	<u>SPHPIMQGVFKWKALDDFKLKNKMDLWKSRTFNNQINKDRFGKIKIGNDV</u>	139
AF462019_1	116	WIGTEAMIMPVKIGHGAIASRSVTKDVAPYEVVGSNPAKHIFRFSD	165
		.. . .   ... ... ... ... ... ... ... ... ... ...	
CLH66_17905	140	<u>WIGEGAFIRRGVKIGDGAIASHSVSKDVAPYSIVGGVPAKHIFRFED</u>	189
AF462019_1	166	VEIAMLLEMAWNNWPESWL-----KESMQSLCSDSIEG-----	198
		.. . . : : ...: ...   ...: ... .	
CLH66_17905	190	<u>NIEQLIEIKWWDYGLSALEGVDFTDINKAIKKIRENIDSGVAEKFTPNV</u>	239
AF462019_1	199	LYLNWQSKART- 209	
		:: ... ..	
CLH66_17905	240	IRINKDGVAEKI 251	

**Fig. S2 (c)** A global protein alignment between the putative novel phenicol inactivating enzyme in *E. coli* CCUG 70745 (PRJNA401330:CLH66\_17905) and the closest match in ResFinder, CatB9 (accession number AF62019) with 31.6% alignment identity, 42.6% similarity, and 24.7% gaps. Underlined region corresponds to the match against the conserved domain “Zenobiotic acyltransferase (XAT)” (see FIG. S2d).

		10	20	30	40	50	60	70	80	
		...*... ...*... ...*... ...*... ...*... ...*... ...*... ...*...								
CLH66_17905	48	<u>IGAFSYMGGKSTHMLHI-ESIGRFCAIAGNVVAGA-MEHPAHFLSPHPIMQGVFKWKALDDFKlknkDMLWksrtfnqi</u>	125							
Cdd:cd03349	4	<u>VGDYSYSGPDCDVGdKLSIGKFCISIApGVKIGlgNHPDtwvSTYPFYIFGGEWEDDAKFD----</u>	71							
		90	100	110	120	130	140	150		
		...*... ...*... ...*... ...*... ...*... ...*... ...*... ...*...								
CLH66_17905	126	<u>nkdrfGKIKIGNDVWIGEGAFIRRGVKIGDGAIASHSVSKDVAPYSIVGGVPAKHIFRFEDNIEQLIEIKWWDY</u>	203							
Cdd:cd03349	72	<u>-----GDVIIGNDVWIGGATILpGVtIGDGAIVAGAVTKDVPYAIvGGNPAKVIRYRFDEETIERLLALKWWDW</u>	144							

**FIG. S2 (d)** Local alignment between the putative novel phenicol inactivating enzyme in *E. coli* CCUG 70745 (PRJNA401330:CLH66\_17905) and the closest matching domain in the Conserved Domains Database (CDD): Zenobiotic acyltransferase (XAT) (Accession: cd03349; pssm-ID: 100040; domain length: 145; e-value: 6.30e-54). “The XAT class of hexapeptide acyltransferases is composed of a large number of microbial enzymes that catalyze the CoA-dependent acetylation of a variety of hydroxyl-bearing acceptors such as chloramphenicol and streptogramin, among others.”