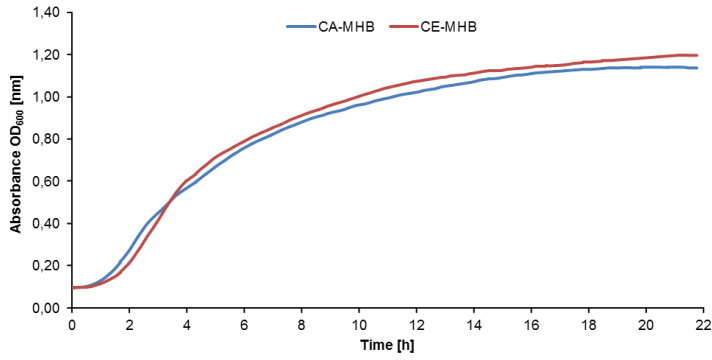


1 **Appendix**

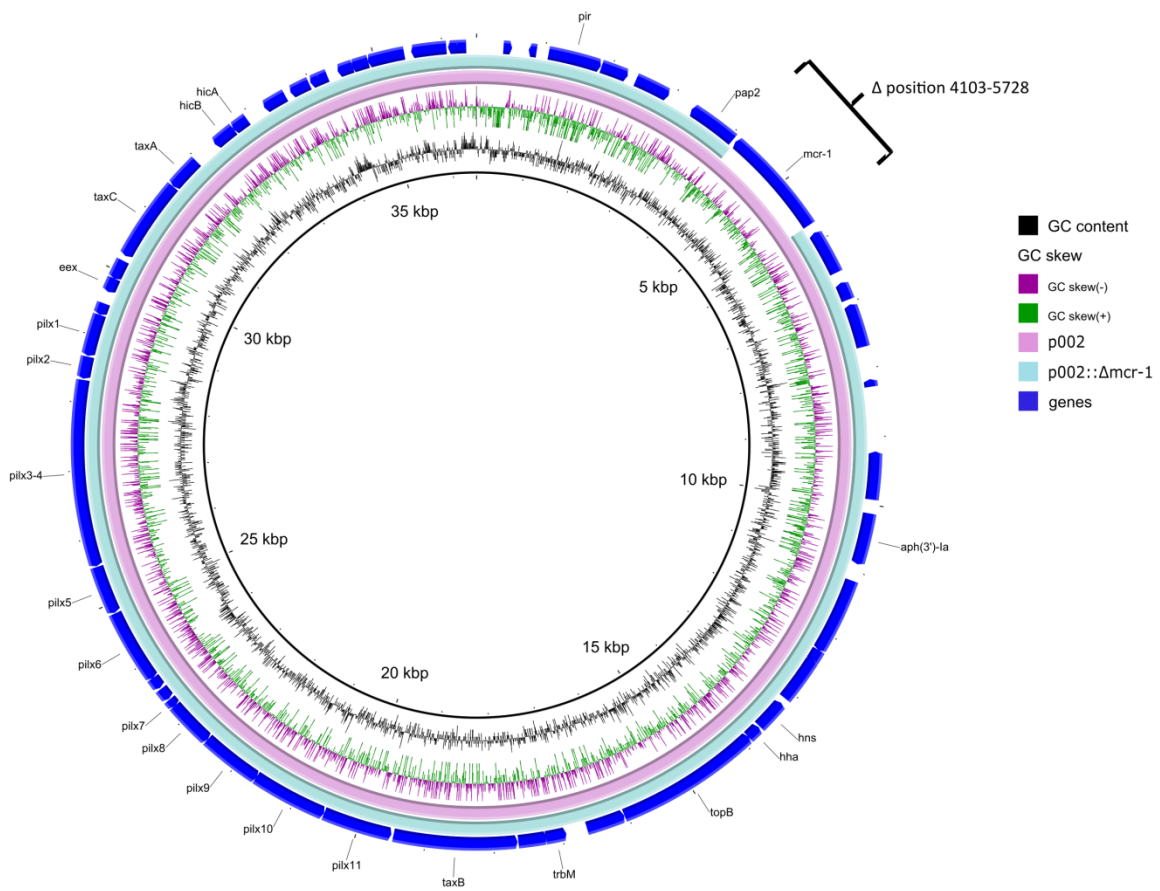
2 **Figures**

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5 Figure A1. Growth curves of representative *E. coli* in the cation-adjusted Mueller-Hinton (CA-
6 MH) broth and cation-enhanced Mueller-Hinton (CE-MH) broth. The concentration of calcium
7 present in CE-MH has no adverse effects on bacterial growth.



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9 Figure A2 Map of *mcr-I*-encoding IncX4 plasmids used in the study. p002 and p002::*Δmcr-I* have
 10 identical sequences apart from the absence of the *mcr-I* gene in the latter plasmid (marked with
 11 the bracket).

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17 **Tables**

18 Table A1 The colistin MIC susceptibility for a subset of isolates encoding the *mcr-1* gene
 19 determined by microdilution method in the reference cation-adjusted Mueller Hinton (CA-MH)
 20 broth containing 20% of heat-inactivated normal human serum (iNHS).

Isolate (Inc group)	Broth microdilution MIC* [mg/L]	
	CA-MH	CA-MH supplemented with 20% NHS
<i>E. coli</i> _001 (IncHI2)	4	6
<i>E. coli</i> _002 (IncX4)	4	6
<i>E. coli</i> _006 (IncX4)	4	6
<i>E. coli</i> _018 (chromosomal)	4	6
<i>E. coli</i> _055 (ND)	4	6
<i>E. coli</i> DO14** (NA)	0.5	0.5
<i>E. coli</i> DO21** (NA)	0.5	0.5
<i>E. coli</i> _017** (NA)	0.5	0.5

21 *The MIC is defined as the lowest concentration of the colistin that inhibits visible growth of the tested isolate as
 22 observed with the unaided eye, ** *mcr-1*-negative isolate, ND – not determined, NA – not applicable

23 Table A2 Essential and categorical agreements between colistin MIC values obtained in the
 24 reference cation-adjusted Mueller-Hinton (CA-MH) broth and cation-enhanced Mueller-Hinton
 25 (CE-MH) broth determined by broth microdilution (BMD) and Strip-based methods.

	Susceptibility testing method	Colistin-susceptible	Colistin-resistant (n=68)
		(n=32)	
% Essential agreement (EA) ^a	BMD	91	24
	Strip-based	100	13
% Categorical agreement (CA) ^b	BMD	91	99
	Strip-based	100	87

26 ^a MICs being within ± 1 dilution of reference MICs.

27 ^b Test results with correct susceptibility categorization.

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29 Table A3 The colistin quality control results for broth microdilution and strip-based methods in
 30 calcium-enhanced Mueller-Hinton (CE-MH) broth performed according to the 15 replicate (3 x 5
 31 day) plan.

Colistin MIC method	Colistin MIC [mg/L]										
	<i>E. coli</i> ATCC 25922		<i>E. coli</i> NCTC 13846		<i>E. coli</i> _010		<i>E. coli</i> CLO28		<i>E. coli</i> _051		
	0.5	1	1.5	6	32	1	2	1	1.5	16	32
Broth microdilution	1	14			15	13	2	15		1	14
Strip-based test		2	13	15		1	14	13	2	15	

32
 33 Table A4 The nucleotide sequence of the primer used for introduction of substitution (D149Y) into
 34 *pmrB* gene of *E. coli* MG1655 by pORTMAGE system.

Primer	Sequence 5'→3'
portMAGE_pmrB_D149Y	gtcgctgaccagcagcgtggataacgaaaggtgtttaccgcttacgtcgcgcacgaactgcaacgccactggcgggggtgcgtttgc*

35 * The oligonucleotide contains phosphorothioate linkages at both 5' and 3' termini for protection against endogenous nucleases.

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 37 Table A5 The nucleotide sequences of the primers used for deletion of *mcr-1* gene from IncX4
 38 plasmid p002.

Primer	Sequence 5'→3'
$\Delta mcr-1$ _Fragment1_FOR	ttccatctcaacagatctctgattcgaacc
$\Delta mcr-1$ _Fragment1_REV	ttgtctgtttcgaaaagattatcgtggattgt
$\Delta mcr-1$ _Fragment2_FOR	gttataacaatccacgataatctttcgaaaacagaca
$\Delta mcr-1$ _Fragment2_REV	tatttttgagtagtttctcttctccctgtatttttccaaaccacc
$\Delta mcr-1$ _Fragment3_FOR	ggaaaaatacagggagaaagagaactactcaaaaaataaacggtggga
$\Delta mcr-1$ _Fragment3_REV	tgggctgtggttcgaatcagagatctgttga

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