© 2021 Wiley-VCH GmbH



Supporting Information

for Global Challenges, DOI: 10.1002/gch2.202000014

Comprehensive Genomic Investigation of Coevolution of *mcr* genes in *Escherichia coli* Strains via Nanopore Sequencing

Ruichao Li, Pengcheng Du, Pei Zhang, Yan Li, Xiaorong Yang, Zhiqiang Wang, Juan Wang, and Li Bai*

Supplementary Data

Comprehensive genomic investigation of coevolution of *mcr* genes in *E. coli* strains via nanopore sequencing

Ruichao Li^{1,2}[†], Pengcheng Du³[†], Pei Zhang⁴, Yan Li¹, Xiaorong Yang⁵, Zhiqiang Wang¹, Juan Wang⁶, Li Bai⁴*

¹ Jiangsu Co-Innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, College of Veterinary Medicine, Yangzhou University, Yangzhou, Jiangsu, P. R. China;

² Institute of Comparative Medicine, Yangzhou University, Yangzhou, Jiangsu, P. R. China;

³ Institute of Infectious Diseases, Beijing Ditan Hospital, Capital Medical University, and Beijing Key Laboratory of Emerging Infectious Diseases, Beijing, P. R. China;

⁴ Key Laboratory of Food Safety Risk Assessment, National Health Commission of the People's Republic of China, China National Center for Food Safety Risk Assessment, Beijing, P. R. China;

⁵ Center for Disease Control and Prevention of Sichuan Province, Chengdu, Sichuan, P. R. China;

⁶College of Veterinary Medicine, Northwest A&F University, Yangling, Shaanxi, P. R. China.

Running title: Characterization of bacterial populations harboring mcr-1 and mcr-3 genes

†Contributed equally to the work

*Corresponding author: Li Bai, Tel: 8610-52165543; Email: baili@cfsa.net.cn

China National Center for Food Safety Risk Assessment, Beijing, China

Key words: colistin resistance, plasmids, mcr genes, coevolution

Strains ^a	Antimicrobials ^b														
	СТХ	CAZ	IMP	TET	CFZ	CFX	GEN	AMP	AMS	CHL	CIP	SXT	NAL	AZI	СТ
CP8-3	≤0.25	≤1	≤0.25	>32	2	8	>32	>64	8	>64	1	>8/152	16	16	8
CP8-3-T	≤0.25	≤0.5	≤0.25	32	4	8	32	>64	8/4	>64	0.5	≤0.25/4.75	16	≤2	4
CP55	≤0.25	≤0.5	≤0.25	>32	4	8	16	>64	8/4	>64	0.25	≤0.25/4.75	8	≤2	8
CP55-T1	≤0.25	≤0.5	≤0.25	4	4	16	≤1	8	8/4	8	≤0.03	≤0.25/4.75	≤4	≤2	4
CP55-T2	≤0.25	≤0.5	≤0.25	4	2	4	≤1	4	4/2	8	≤0.03	≤0.25/4.75	≤4	4	4
CP61	≤0.25	≤1	≤0.25	>32	4	8	>32	>64	8	>64	0.5	>8/152	8	8	4
CP66-6	0.5	≤1	1	>32	16	32	>32	>64	>64	>64	32	>8/152	>64	>64	8
CP131	≤0.25	≤1	≤0.25	>32	8	16	>32	>64	16	>64	2	>8/152	>64	16	4

Table S1. Antimicrobial susceptibility testing of different *E. coli* strains and their transconjugants against different drugs (MICs, µg/mL).

^a The strains with the letter T were the corresponding transconjugants with *E. coli* J53 as the recipient strain.

^b Drugs abbreviations: CTX, cefotaxime; CAZ, ceftazidime; IMP, imipenem; TET, tetracycline; CFZ, cefazolin; CFX, cefoxitin; GEN, gentamycin; AMP, ampicillin; AMS, ampicillin/sulbactam; CHL, chloramphenicol; CIP, ciprofloxacin; SXT, trimethoprim/sulfamethoxazole; NAL, nalidixic acid; AZI, azithromycin; CT, colistin.

		CP131	CP55	CP61	CP66-6	CP8-3
Ol(Ye444)05 1_F15994 0 0 0 0 81.026 Col(F2_1_NC_009781 0 98.026 0 0 0 Col(F2_1_N205800 0 0 0 0 0 DI(NAL_1_DQ298019 0 89.256 0 0 0 IS1006 0 0 94.896 0 0 IS102 0 0 94.896 0 0 IS102 0 0 94.896 0 0 IS1203 0 95.42 0 99.763 99.763 IS150 99.861 0 99.792 0 99.861 IS15DI 0 91.46 0 0 99.512 IS15DI 0 100 0 0 99.525 IS184 90.625 0 90.625 0 98.564 IS181 94.01 99.219 0 90.625 0 IS181 94.01 99.219	Col(MG828)_1NC_00 8486	0	91.603	0	0	0
Coll56_1_NC_009781 0 98.026 0 0 0 ColKP3_1_JN205800 0 0 0 0 0 0 OIRNAI_1_DQ298019 0 89.256 0 0 0 IS1006 0 0 99.878 0 0 IS102 0 0 94.896 0 0 IS102 0 95.42 0 95.42 0 IS1203 0 95.42 0 99.763 99.763 IS150 99.861 0 99.792 0 99.861 IS15D 0 91.46 0 0 99.512 IS15DIV 0 100 0 0 99.925 IS14 90.625 0 98.429 0 0 0 IS14 90.625 0 98.429 0 0 0 98.957 IS14 91.021 99.219 0 90.625 0 0 0	Col(Ye4449)_1FJ6964 05	0	0	0	0	81.026
ColKP3N205800 0 0 100 0 olRNAL1_DQ298019 0 89.256 0 0 0 IS1006 0 0 94.896 0 0 IS102 0 0 94.896 0 0 IS102 0 0 95.42 0 95.42 0 IS1203 0 95.42 0 97.63 99.763 99.763 IS1204 0 0 99.763 99.763 99.763 99.763 IS150 99.861 0 99.792 0 99.861 99.925 IS15D 0 90.01 100 0 0 99.925 IS1A 90.625 0 98.957 0 0 0 98.957 IS1R 94.01 99.219 0 90.625 0 0 IS1SD 0 0 98.827 98.564 98.957 0 0 0 IS1SD 0 <td>Col156_1NC_009781</td> <td>0</td> <td>98.026</td> <td>0</td> <td>0</td> <td>0</td>	Col156_1NC_009781	0	98.026	0	0	0
olrNAL1_DQ298019 0 89.256 0 0 IS1006 0 0 99.878 0 IS102 0 0 94.896 0 0 IS102 0 95.42 0 95.42 0 IS1203 0 95.42 0 97.63 99.763 IS1294 0 0 99.792 0 99.861 IS150 99.861 0 99.792 0 99.512 IS15DIV 0 100 0 0 0 IS186B 99.925 99.925 99.925 0 98.958 IS14 90.625 0 96.597 0 0 0 IS14 0 98.56 0 98.429 0 IS17 0 0 0 97.763 0 0 IS18 94.01 99.219 0 98.64 98.957 0 0 0 0 0 0 0	ColKP3_1JN205800	0	0	0	100	0
IS1006 0 0 99.878 0 IS102 0 0 94.896 0 0 IS1203 0 95.42 0 95.42 0 IS1203 0 95.42 0 95.42 0 IS1294 0 0 99.763 99.763 IS150 99.861 0 99.792 0 99.861 IS15DI 0 99.146 0 0 99.512 IS15DIV 0 100 0 0 99.925 IS15DIV 0 100 0 0 99.925 IS14 90.625 0 98.958 1515 0 0 0 IS1R 90.625 0 98.957 0 0 0 0 IS1R 94.01 99.219 0 96.625 0 0 0 IS1R 94.01 99.219 98.5672 95.492 0 0 0 0	ColRNAI_1DQ298019	0	89.256	0	0	0
IS1020094.89600IS1203095.42095.420IS129400099.76399.763IS15099.861099.792099.861IS15D1099.146000IS15D1V0100000IS16B899.92599.92599.925099.925IS1A90.625090.625098.958IS1F096.597000IS1R94.0199.219090.6250IS1SD0098.82798.56498.957IS1X2000097.786IS295.64296.09395.67295.4920IS200089.255000IS2000099.92199.9210IS2000099.83699.91899.54599.836IS3099.83699.83699.91899.54599.836IS3000000000IS3000000000IS575097.59797.42697.65497.712IS6100000000IS62999.54297.7860099.446	IS1006	0	0	0	99.878	0
IS1203 0 95.42 0 95.42 0 IS1294 0 0 0 99.763 99.763 IS150 99.861 0 99.792 0 99.861 IS15D1 0 99.146 0 0 99.512 IS15D1V 0 100 0 0 0 IS186B 99.925 99.925 99.925 0 99.925 IS1A 90.625 0 90.625 0 98.958 IS1F 0 96.597 0 0 0 IS1H 0 98.56 0 98.429 0 IS1F 0 96.597 0 0 0 IS1R 94.01 99.219 0 90.625 0 IS1SD 0 0 98.827 98.564 98.957 IS1X2 0 0 0 0 0 IS200C 0 0 0 0 0 </td <td>IS102</td> <td>0</td> <td>0</td> <td>94.896</td> <td>0</td> <td>0</td>	IS102	0	0	94.896	0	0
IS12940099.76399.763IS15099.861099.792099.861IS15DI099.1460099.512IS15DIV0100000IS186B99.92599.92599.925099.925IS1A90.625090.625098.958IS1F096.597000IS1R94.0199.219090.6250IS1SD0098.82798.56498.957IS1X200097.786IS295.64296.09395.67295.4920IS200089.255000IS2699.878099.63410099.616IS3099.81699.83699.91899.54599.836IS300000000IS421R099.548000IS50500000IS609097.59797.42697.65497.712IS610000000IS62999.54297.786000	IS1203	0	95.42	0	95.42	0
IS150 99.861 0 99.792 0 99.861 IS15DI 0 99.146 0 0 99.512 IS15DIV 0 100 0 0 99.925 IS186B 99.925 99.925 99.925 0 98.958 IS1A 90.625 0 90.625 0 98.958 IS1F 0 96.597 0 0 0 IS1F 0 98.56 0 98.429 0 IS1R 94.01 99.219 0 90.625 0 IS1SD 0 0 98.827 98.564 98.957 IS1X2 0 0 0 97.786 0 IS200 0 89.255 0 0 0 IS200 0 99.634 100 99.636 IS200 0 0 99.634 99.836 99.836 IS30 99.836 99.921 99.921 99.836	IS1294	0	0	0	99.763	99.763
IS15DI 0 99.146 0 0 99.512 IS15DIV 0 100 0 0 0 IS186B 99.925 99.925 99.925 0 99.925 IS1A 90.625 0 90.625 0 98.958 IS1F 0 96.597 0 0 0 IS1F 0 98.56 0 98.429 0 IS1R 94.01 99.219 0 90.625 0 IS1R 94.01 99.219 0 90.625 0 IS1R 94.01 99.219 0 98.564 98.957 IS1SD 0 0 0 97.786 0 IS200 0 89.255 0 0 0 IS200 0 89.255 0 0 0 IS200 0 0 99.541 99.921 99.921 0 IS30 99.836 99.836 99.918	IS150	99.861	0	99.792	0	99.861
IS15DIV 0 100 0 0 99.925 IS186B 99.925 99.925 99.925 0 98.958 IS1A 90.625 0 98.958 0 98.958 IS1F 0 96.597 0 0 0 IS1H 0 98.56 0 98.429 0 IS1R 94.01 99.219 0 90.625 0 IS1SD 0 0 98.827 98.564 98.957 IS1SD 0 0 0 0 97.786 IS2 95.642 96.093 95.672 95.492 0 IS200 0 89.255 0 0 0 IS200C 0 0 99.577 0 0 IS200C 0 0 99.545 99.836 0 0 0 IS30 99.836 99.836 99.921 99.921 0 0 0 0 0 <td< td=""><td>IS15DI</td><td>0</td><td>99.146</td><td>0</td><td>0</td><td>99.512</td></td<>	IS15DI	0	99.146	0	0	99.512
IS186B99.92599.92599.925099.925IS1A90.625090.625098.958IS1F096.597000IS1H098.56098.4290IS1R94.0199.219090.6250IS1SD0098.82798.56498.957IS1X2000097.786IS295.64296.09395.67295.4920IS200C00099.5770IS2699.878099.62410099.616IS399.92199.92199.92199.9210IS30000000IS4321R099.548000IS5075099.623000IS609097.59797.42697.65497.712IS61000001000IS62999.54297.7860099.846	IS15DIV	0	100	0	0	0
IS1A90.625090.625098.958IS1F096.597000IS1H098.56098.4290IS1R94.0199.219090.6250IS1SD0098.82798.56498.957IS1X2000097.786IS295.64296.09395.67295.4920IS200089.255000IS200C0099.5770IS2699.878099.62310099.616IS399.92199.92199.92199.9210IS30000000IS4321R00000IS5075097.59797.42697.65497.712IS609097.59797.42697.65497.712IS610000000IS62999.54297.7860099.846	IS186B	99.925	99.925	99.925	0	99.925
IS1F096.597000IS1H098.56098.4290IS1R94.0199.219090.6250IS1SD0098.82798.56498.957IS1X2000097.786IS295.64296.09395.67295.4920IS200089.255000IS200C0099.5770IS2699.878099.63410099.616IS399.92199.92199.92199.9210IS30000000IS4321R099.548000IS5075097.59797.42697.65497.712IS6090001000IS610000099.8450IS621099.923099.8450	IS1A	90.625	0	90.625	0	98.958
IS1H098.56098.4290IS1R94.0199.219090.6250IS1SD0098.82798.56498.957IS1X2000097.786IS295.64296.09395.67295.4920IS200089.255000IS200C0099.5770IS2699.878099.63410099.616IS399.92199.92199.92199.9210IS3099.83699.83699.91899.54599.836IS3000000000IS4321R099.6230000IS5075097.59797.42697.65497.712IS6090001000IS610000099.8450IS621099.54297.7860099.466	IS1F	0	96.597	0	0	0
IS1R94.0199.219090.6250IS1SD0098.82798.56498.957IS1X2000097.786IS295.64296.09395.67295.4920IS200089.255000IS200C0099.5770IS2699.878099.63410099.616IS399.92199.92199.92199.9210IS3099.83699.83699.91899.54599.836IS3000000000IS4321R099.548000IS5075097.59797.42697.65497.712IS6090001000IS610000099.923099.845IS621099.7860099.466	IS1H	0	98.56	0	98.429	0
IS1SD0098.82798.56498.957IS1X2000097.786IS295.64296.09395.67295.4920IS200089.255000IS200C00099.5770IS2699.878099.63410099.616IS399.92199.92199.92199.9210IS3099.83699.83699.91899.54599.836IS300000000IS5500000IS5075097.59797.42697.65497.712IS610000000IS621099.923099.8450IS62999.54297.7860099.466	IS1R	94.01	99.219	0	90.625	0
IS1X2000097.786IS295.64296.09395.67295.4920IS200089.255000IS200C00099.5770IS2699.878099.63410099.616IS399.92199.92199.92199.9210IS3099.83699.83699.91899.54599.836IS300000099.8660IS4321R099.548000IS5075099.623000IS609097.59797.42697.65497.712IS610000000IS621099.923099.8450IS62999.54297.7860099.466	IS1SD	0	0	98.827	98.564	98.957
IS295.64296.09395.67295.4920IS200089.255000IS200C00099.5770IS2699.878099.63410099.616IS399.92199.92199.92199.9210IS3099.83699.83699.91899.54599.836IS300000099.8660IS4321R099.623000IS5075097.59797.42697.65497.712IS60900000IS621099.923099.8450IS62999.54297.7860099.466	IS1X2	0	0	0	0	97.786
IS200089.255000IS200C0099.5770IS2699.878099.63410099.616IS399.92199.92199.92199.9210IS3099.83699.83699.91899.54599.836IS300000099.8660IS4321R099.548000IS500000IS5075099.623000IS609097.59797.42697.65497.712IS61000001000IS621099.923099.8450IS62999.54297.7860099.466	IS2	95.642	96.093	95.672	95.492	0
IS200C0099.5770IS2699.878099.63410099.616IS399.92199.92199.92199.9210IS3099.83699.83699.91899.54599.836IS300000099.8660IS4321R099.548000IS500000IS5075099.623000IS609097.59797.42697.65497.712IS610000000IS621099.923099.8450IS62999.54297.7860099.466	IS200	0	89.255	0	0	0
IS2699.878099.63410099.616IS399.92199.92199.92199.9210IS3099.83699.83699.91899.54599.836IS300000099.8660IS4321R099.548000IS500000IS5075099.623000IS609097.59797.42697.65497.712IS610000000IS621099.923099.8450IS62999.54297.7860099.466	IS200C	0	0	0	99.577	0
IS399.92199.92199.92199.92199.9210IS3099.83699.83699.91899.54599.836IS300000099.8660IS4321R099.548000IS500000IS5075099.623000IS609097.59797.42697.65497.712IS610000000IS621099.923099.8450IS62999.54297.7860099.466	IS26	99.878	0	99.634	100	99.616
IS3099.83699.83699.91899.54599.836IS300000099.8660IS4321R099.548000IS500000IS5075099.623000IS609097.59797.42697.65497.712IS61000001000IS621099.923099.8450IS62999.54297.7860099.466	IS3	99.921	99.921	99.921	99.921	0
IS30000099.8660IS4321R099.548000IS500000IS5075099.623000IS609097.59797.42697.65497.712IS61000001000IS621099.923099.8450IS62999.54297.7860099.466	IS30	99.836	99.836	99.918	99.545	99.836
IS4321R099.548000IS500000IS5075099.623000IS609097.59797.42697.65497.712IS61000001000IS621099.923099.8450IS62999.54297.7860099.466	IS3000	0	0	0	99.866	0
IS500000IS5075099.623000IS609097.59797.42697.65497.712IS61000001000IS621099.923099.8450IS62999.54297.7860099.466	IS4321R	0	99.548	0	0	0
IS5075099.623000IS609097.59797.42697.65497.712IS61000001000IS621099.923099.8450IS62999.54297.7860099.466	IS5	0	0	0	0	0
IS609097.59797.42697.65497.712IS61000001000IS621099.923099.8450IS62999.54297.7860099.466	IS5075	0	99.623	0	0	0
IS61000001000IS621099.923099.8450IS62999.54297.7860099.466	IS609	0	97.597	97.426	97.654	97.712
IS621099.923099.8450IS62999.54297.7860099.466	IS6100	0	0	0	100	0
IS629 99.542 97.786 0 0 99.466	IS621	0	99.923	0	99.845	0
	IS629	99.542	97.786	0	0	99.466
IS903B 95.845 0 98.297 89.225 96.975	IS903B	95.845	0	98.297	89.225	96.975

Table S2. Distribution of resistance genes, insertion sequences and replicon genes among thefive *E. coli* genomes positive for *mcr-1* and *mcr-3* genes.

IS911	94.052	0	0	0	0
ISAba1	0	0	0	0	0
ISApl1	99.907	0	99.907	99.907	99.907
ISCro1	98.111	0	0	0	0
ISCro3	0	0	0	90.756	0
ISEc1	0	96.897	96.406	96.664	95.279
ISEc12	99.961	0	0	0	0
ISEc17	0	0	0	0	99.921
ISEc23	0	96.327	0	0	0
ISEc26	0	98.677	98.511	98.677	0
ISEc31	0	92.732	0	0	0
ISEc32	0	100	0	0	0
ISEc39	0	99.62	0	0	0
ISEc5	90.495	0	0	0	0
ISEc8	0	99.14	85.003	0	85.003
ISErsp1	0	88.436	0	0	0
ISKpn26	96.739	0	0	0	99.582
ISSso1	0	87.215	0	0	0
ISSty2	0	0	81.445	0	0
ISSwi1	0	0	0	0	0
ISVsa3	0	0	0	100	0
ISVsa5	0	0	98.947	99.925	0
IncB/O/K/Z_2_GU25664	0	95.484	0	0	0
IncFIA(HI1)_1_HI1_AF 250878	0	0	98.711	0	98.711
IncFIB(AP001918)_1 AP001918	98.827	98.507	0	97.678	0
IncFIB(K)_1_Kpn3_JN2 33704	0	0	98.929	0	98.929
IncFIB(pHCM2)_1_pHC M2_AL513384	0	0	0	0	0
IncFIC(FII)_1_AP0019	94.188	0	0	94	0
IncFII(pSE11)_1_pSE11 _AP009242	0	94.34	0	0	0
IncFII_1_AY458016	0	100	0	100	100
IncHI1A_1AF250878	0	0	0	0	0
IncHI1B(R27)_1_R27_A F250878	0	0	0	0	0
IncN_1_AY046276	0	0	100	0	100
IncQ1_1_HE654726	0	0	0	96.222	100
IncR_1DQ449578	0	0	100	0	100

IncX1_1_EU370913	0	0	98.663	0	98.93
IncX3_1JN247852	0	0	0	100	0
IncX4_1CP002895	0	100	0	0	0
IncY_1K02380	0	0	0	0	0
QnrS1_1_AB187515	0	100	0	100	100
QnrS2_1_JF261185	0	0	100	0	0
aac(3)-IId_1_EU022314	0	99.884	0	99.884	0
aac(3)-VIa_1_M88012	0	0	99.778	0	99.778
aadA1_1_X02340	0	0	89.5	89.5	89.625
aadA2_2_JQ364967	100	0	0	0	0
aph(3')-Ia_1_V00359	0	0	100	100	0
blaOXA-181_1_HM9929 46	0	0	0	100	0
blaTEM-1B_1_JF910132	100	100	100	99.884	100
catA1_1_V00622	0	99.848	0	0	0
cmlA1_1_M64556	0	0	99.921	99.921	99.841
dfrA12_1_AB571791	100	0	100	100	100
erm(B)_18_X66468	0	0	0	0	0
floR_2_AF118107	98.188	98.188	98.188	98.105	98.188
fosA_14_AB522970	0	0	0	91.367	0
lnu(F)_1_EU118119	0	0	0	100	0
mcr-1_1_KP347127	100	100	100	100	100
mcr3_NG_055505	0	99.831	100	99.831	99.831
mef(B)_1_FJ196385	0	0	100	0	100
mph(A)_2_U36578	99.675	99.636	0	99.566	0
strA_4_AF321551	0	99.876	0	0	0
strB_1_M96392	0	99.881	0	0	0
sul2_2_GQ421466	0	100	0	100	0
sul3_2_AJ459418	100	0	100	100	100
tet(A)_4_AJ517790	99.917	100	99.917	99.917	99.917
tet(M)_4_X75073	0	0	0	0	99.896
tet(M)_8_X04388	96.146	0	96.146	96.146	96.146

Table S3. Genetic characterization of *mcr-1* and *mcr-3* genes among the five strains in this study.

Strains	mcr-1 location	<i>mcr-1</i> structures	mcr-3 genes	mcr-3 location	<i>mcr-3</i> structures

CP8-3	chromosome	ISApl1-mcr-1- <i>Apap2</i> -IS1294- ISApl1	mcr-3.5	IncR plasmid	qnrS1-ISKpn19-TnAs2-mcr-3.5-dgkA-IS15-TnAs2
CP55	IncX4 plasmid	mcr-1-pap2	mcr-3.5	IncFII plasmid	IS4321R-TnAs2-mcr-3.5-dgkA-IS15
CP61	chromosome	ISApl1- mcr-1- pap2- ISApl1	mcr-3.1	IncFIB plasmid	ISEc78-TnAs2-mcr-3.1-dgkA-ISKpn40
CP66-6	chromosome	ISApl1- mcr-1- 4pap2-IS1294- ISApl1	mcr-3.5	IncFII plasmid	bla _{TEM-1B} -TnAs2-mcr-3.5-dgkA-IS26
CP131	chromosome	ISApl1- mcr-1- pap2- ISApl1	mcr-3.1	IncFIB plasmid	aac(3)-IId-IS1R-TnAs2-mcr-3.1-dgkA-ISKpn40

Table S4. The ratios of subclones losing *mcr-1* or/and *mcr-3* genes among the five *E. coli* strains during bacterial passages last for 28 days. P denotes the serial cultural passage day (1, 7, 14, 21 and 28). The denominators represent the average total colony numbers in the agar plates, and the numerators for the numbers of colonies losing the *mcr* genes confirmed by PCR. All the ratios in red show the passaged cultures with colonies losing *mcr* genes.

Strains		Loss o	of <i>mcr-1</i> with	out colistin		Loss of <i>mcr-3</i> without colistin						
	Clone_l oss_P1	Clone_los s_P7	Clone_loss _P14	Clone_loss _P21	Clone_loss _P28	Clone_los s_P1	Clone_los s_P7	Clone_loss _P14	Clone_loss _P21	Clone_loss _P28		
CP8-3	0/158	0/210	0/146	24/114	28/159	4/158	0/210	0/146	0/114	0/159		
CP55	0/123	0/201	0/133	0/152	0/78	4/123	0/201	16/133	20/152	4/78		
CP61	0/92	0/131	8/77	4/48	12/80	8/92	0/131	24/77	44/48	48/80		
CP66-6	0/120	20/211	20/167	28/114	40/210	0/120	32/211	48/167	48/114	48/210		
CP131	0/83	0/101	0/196	4/112	0/105	0/83	0/101	0/196	0/112	4/105		
C		Loss	of <i>mcr-1</i> wi	th colistin		Loss of <i>mcr-3</i> with colistin						
Strains	Clone_l oss_P1	Clone_los s_P7	Clone_loss _P14	Clone_loss _P21	Clone_loss _P28	Clone_los s_P1	Clone_los s_P7	Clone_loss _P14	Clone_loss _P21	Clone_loss _P28		
CP8-3	0/138	0/220	0/200	0/150	0/131	0/138	0/220	0/200	0/150	0/131		
CP55	0/64	0/248	0/83	0/109	0/102	0/64	0/248	0/83	0/109	0/102		
CP61	0/148	0/138	0/105	0/60	0/61	4/148	0/138	0/105	0/60	4/80		
CP66-6	0/100	0/168	0/142	0/75	0/45	0/100	8/168	44/142	40/75	45/45		
CP131	0/45	0/112	0/65	0/52	12/50	0/45	0/112	0/65	0/52	0/50		



Figure S1. S1-PFGE of the original five *mcr***-bearing** *E. coli* **strains.** The first lane indicates the plasmid weight markers (S1-nuclease digested 39R). CP53 was not included in this study.



Figure S2. S1-PFGE of two *mcr*-bearing *E. coli* strains CP8-3 and CP55, and their transconjugants. Lane1: S1-nuclease digested 39R as a molecular weight marker; Lanes2: CP8-3 (donor), Lanes3: CP8-3-T (transconjugant), Lane5: CP55 (donor), Lane6: CP55-T1 (transconjugant), Lane7: CP55-T2 (transconjugant).



Figure S3. Linear alignment of two IncR *mcr-3*-bearing plasmids with similar backbone structures.



Figure S4. Circular alignment of *mcr-3.1*-bearing plasmid pCP61-92k with other similar plasmids in NCBI database.



Figure S5. Circular alignment of *mcr-3.5*-bearing plasmid pCP66-6-74kb with other similar plasmids in this study and NCBI database.