

# Isolation and characterization of a novel *mcr-5* carrying *Escherichia coli* plasmid from chicken feces in Germany

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## Introduction

Colistin is considered as an important antibiotic of the last resort against human infections with multidrug-resistant (MDR) Gram-negative bacteria. The emergence and spread of mobilizable colistin resistance in Enterobacteriaceae is associated with major public health concerns. Since 2015, several mobile colistin resistance genes were described coding for enzymes of the phosphoethanolamine-transferase family. Nowadays, eight different *mcr*-genes (*mcr-1* to *-8*) were identified, mediating resistances to colistin in different bacterial genera (especially in Enterobacteriaceae). In this study, a novel *mcr-5* carrying plasmid of a commensal *E. coli* recovered from chicken feces is described.

## Basic properties of the *E. coli* isolate and its plasmid

Here we describe a novel *mcr-5* plasmid-prototype from an *E. coli* isolate of the German national monitoring of zoonoses in food and livestock in 2013/2014. The isolate exhibited a MIC against colistin of 4 mg/L as determined by using broth microdilution according to the EUCAST guidelines. Basis genetic features of the Whole Genome Sequencing (WGS) analysis of the isolate are summarized in Table 1. S1-PFGE (Fig. 1) analysis and WGS revealed that the genome of the plasmid pEC1897-13 was 38 kb in size and exhibited 44 putative open reading frames (ORFs; Table 2), respectively.

## Transmissibility of the *mcr-5* carrying plasmid

Bioinformatic analysis indicated that the plasmid belongs to the IncFII group, but represent a novel pMLST-allele that is closely related to the allele FII-82. Beside *mcr-5*, no further resistance determinant was detected on the plasmid.

Interestingly, the plasmid genome of pEC1897-13 obviously comprises all necessary components of a functional IncF conjugative-transfer system (Table 2). However, up to now no self-transmission of the plasmid was observed in *E. coli* by filter mating studies at 37°C under different conditions.

<b>Isolate</b>	13-AB01897
<b>Year of isolation</b>	2013
<b>Source</b>	Chicken, feces
<b>Genome features</b>	
Genome size (bp)	5,256,358
GC content (%)	50.44
<b>Genes (total)</b>	5,758
Genes (coding)	5,317
<b>CDS (total)</b>	5,653
CDS (coding)	5,317
<b>RNA genes (total)</b>	105
rRNAs (total) 5S, 16S, 23S	6, 7, 5
tRNAs	77
ncRNAs	10
<b>Pseudo genes (total)</b>	336
<b>Acquired antimicrobial resistances</b>	
Aminoglycoside	aph(3'')-Ib (100%) aph(6)-Id (100%) aadA1 (100%)
Beta-lactam	blaTEM-1B (100%)
Colistin	mcr-5 (100%)
MLS	mph(B) (100%)
Phenicol	catA1 (99.85%)
Sulphonamide	sul1 (100%) sul2 (100%)
Tetracycline	tet(34) (84.75%, partial) tet(A) (100%)
Trimethoprim	dfrA1 (100%)
<b>Chromosomal point mutations</b>	gyrA S83L
<b>PlasmidFinder (inc group)</b>	
Col	ColRNAI (91.74%, partial) Col(MG828) (94.27%) Col156 (98.03%, partial) ColpVC (98.96%)
IncF	IncFIB(AP001918) (98.39%) IncFII(pCoo) (96.95%)
<b>pMLST</b>	
IncF	F24:A-B1
<b>MLST type <i>E. coli</i> 1</b>	ST-10
<b>MLST type <i>E. coli</i> 2</b>	ST-367
<b>Serotype</b>	H40
<b>FimH type</b>	24

Table 1. Basic features of the 13-AB01897 *E. coli* isolate.

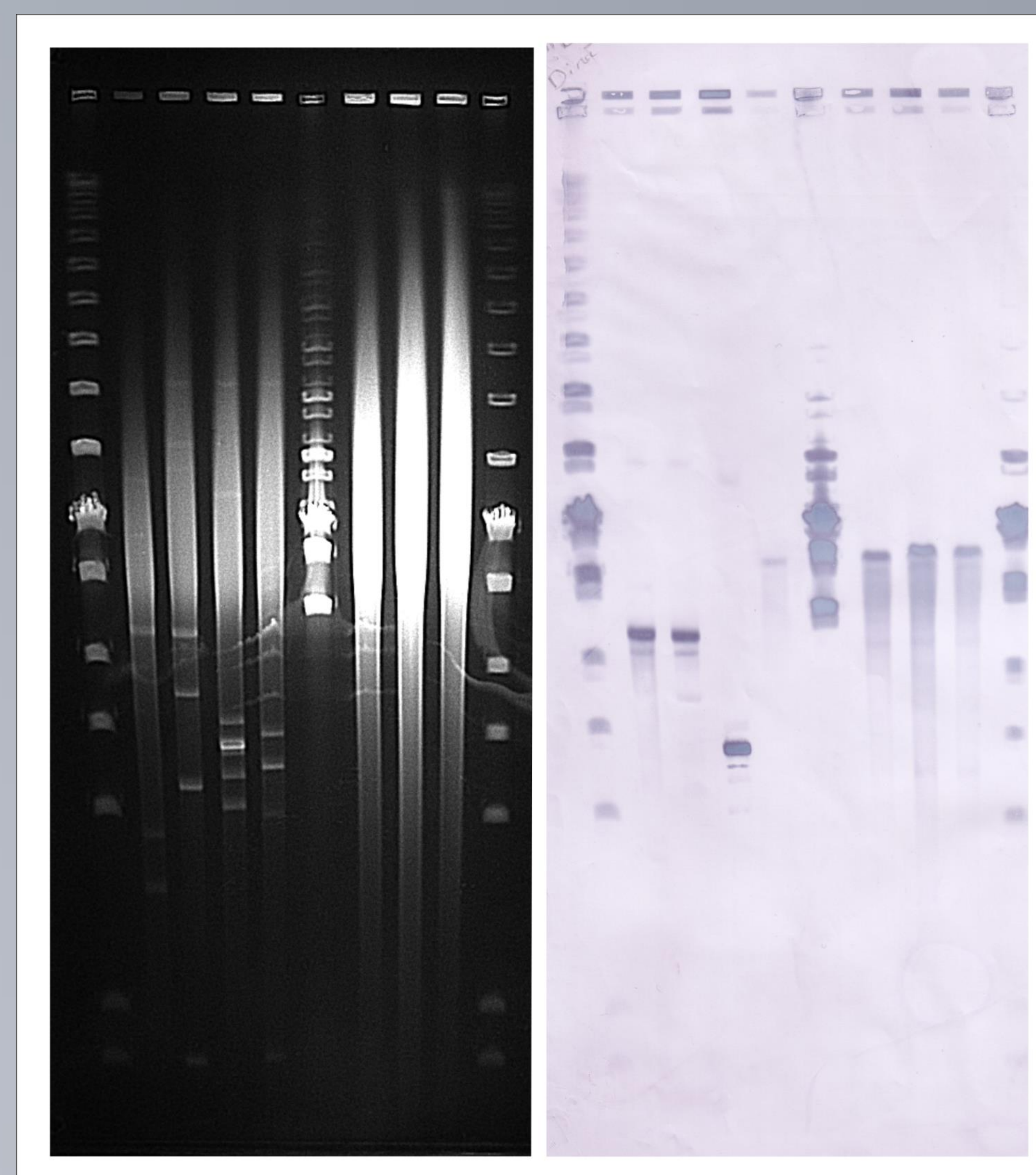


Figure 1. S1-PFGE and DNA-DNA hybridization of *mcr-5* carrying isolates. Lane 1, *S. enterica* 13-SA01718, as well as the *E. coli* isolates 10E01066 (L2), 15-AB00674 (L3), 13-AB01897 (L4) and three different *E. coli* transformants comprising pEC1897-13.

ORF	Start	Stop	Strand	Predicted function
ORF001	1	858	+	Replication protein
ORF002	1558	1698	+	FIG00643981: hypothetical protein
ORF003	4775	1809	-	Mobile element protein
ORF004	5338	4778	-	Mobile element protein
ORF005	5577	6119	+	ChrB domain protein
ORF006	6477	7781	+	Lipid A phosphoethanolamine transferase, putative
ORF007	7778	8338	+	Uncharacterized MFS-type transporter
ORF008	8356	9012	+	Uncharacterized MFS-type transporter
ORF009	9199	9528	+	Toxin HlgB
ORF010	9509	9790	+	Antitoxin HlgA
ORF011	10000	10116	+	hypothetical protein
ORF012	10113	11057	+	hypothetical protein
ORF013	11477	11124	-	stable plasmid inheritance protein B
ORF014	12439	11477	-	Putative stability/partitioning protein
ORF015	12454	12726	+	FIG01048508: hypothetical protein
ORF016	12965	13891	+	FIG01048508: hypothetical protein
ORF017	14168	14043	-	FIG00637984: hypothetical protein
ORF018	14275	15855	+	IncF plasmid conjugative transfer pilus assembly protein TraC
ORF019	15852	16238	+	IncF plasmid conjugative transfer protein TrbI
ORF020	16235	16867	+	IncF plasmid conjugative transfer pilus assembly protein TraW
ORF021	16864	17856	+	IncF plasmid conjugative transfer pilus assembly protein TraU
ORF022	17886	18191	+	Conjugative transfer protein PSLT093
ORF023	18200	18838	+	IncF plasmid conjugative transfer protein TrbC
ORF024	18835	19206	+	FIG00642907: hypothetical protein
ORF025	19232	19654	+	hypothetical protein
ORF026	19651	21501	+	IncF plasmid conjugative transfer protein TraN
ORF027	21525	21785	+	IncF plasmid conjugative transfer protein TrbE
ORF028	21778	22521	+	IncF plasmid conjugative transfer pilus assembly protein TraF
ORF029	22637	22918	+	IncF plasmid conjugative transfer protein TraQ
ORF030	22905	23441	+	IncF plasmid conjugative transfer protein TrbB
ORF031	23938	23435	-	IS1 protein InsB
ORF032	24217	24402	+	IncF plasmid conjugative transfer protein TrbF
ORF033	24389	25762	+	IncF plasmid conjugative transfer pilus assembly protein TraH
ORF034	25759	28233	+	IncF plasmid conjugative transfer protein TraD
ORF035	28233	33503	+	IncF plasmid conjugative transfer DNA-nicking/unwinding protein TraI
ORF036	33523	34269	+	IncF plasmid conjugative transfer pilin acetylase TraX
ORF037	34273	34884	+	IncF plasmid conjugative transfer fertility inhibition protein FinO
ORF038	35016	35228	+	hypothetical protein
ORF039	35515	36192	+	FIG00643845: hypothetical protein
ORF040	36189	36314	+	FIG00643845: hypothetical protein
ORF041	36378	36494	+	hypothetical protein
ORF042	36509	37102	+	YihA
ORF043	37411	37602	+	Replication regulatory protein repA2 (Protein copB)
ORF044	37893	37654	-	hypothetical protein

Table 2. ORF analysis of the pEC1897-13 plasmid genome

## Conclusion

Here we report on a novel IncFII *mcr-5* carrying plasmid-prototype recovered from chicken feces in 2013. The impact of the plasmid for the transmission of colistin resistances is unknown. However, despite of most of the described *mcr-5* carrying plasmids pEC1897-13 carry a complex IncF-like transfer system that may be potentially functional under some specific circumstances.

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