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mcr-5 and a novel *mcr-5.2* variant in *Escherichia coli* isolates from food and food-producing animals, Germany, 2010 to 2017

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Sir,

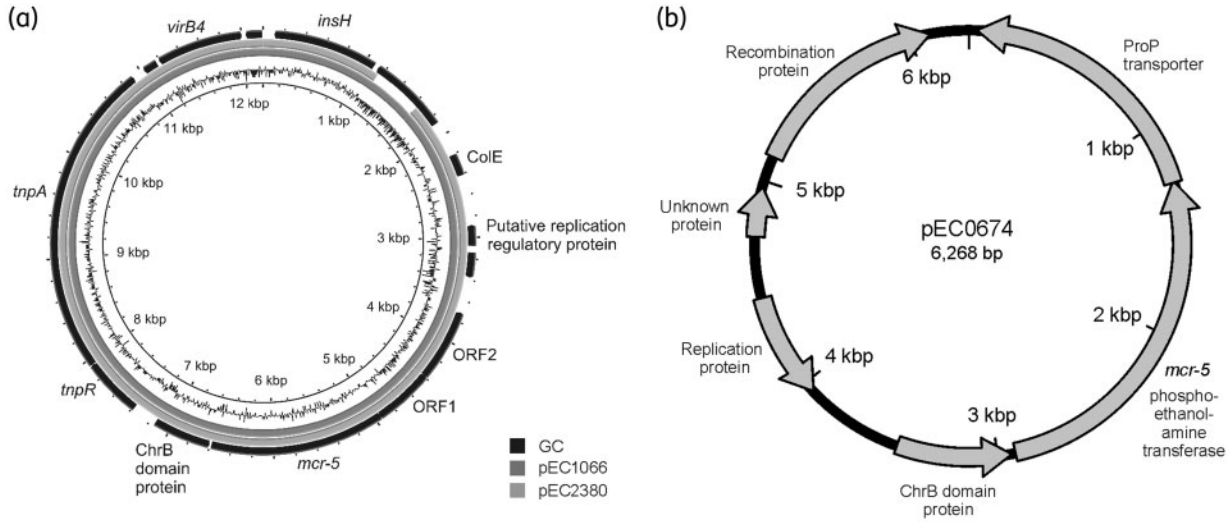
Colistin is considered a last-resort antibiotic used to treat severe human infections caused by MDR Gram-negative bacteria. Thus, spread of colistin resistance among humans would be associated with major public health concerns.[1,2] In 2015, the first mobile colistin resistance gene, *mcr-1*, encoding a phosphoethanolamine transferase enzyme, was identified on a transmissible plasmid.[3] Soon after, *mcr-2* and *mcr-3* were detected on other conjugative plasmids in Enterobacteriaceae.[4,5] Recently, Carattoli *et al.*[6] and Borowiak *et al.*[7] reported two novel genes, *mcr-4* and *mcr-5*, in *Salmonella enterica* serovar Typhimurium and *d*-tartrate-fermenting *S. enterica* serovar Paratyphi B [*Salmonella* Paratyphi B (*d*Ta+)], respectively. Both genes were located on non-conjugative ColE plasmids, either transmissible by a helper plasmid (*mcr-4*) or mobilizable by a Tn*3*-type transposon (*mcr-5*, registered as Tn*6452* on the LSTM website, http://transposon.lstmed.ac.uk/) found on the *Salmonella* Paratyphi B (*d*Ta+) chromosome and plasmids.[7] Identification of different *mcr* genes in Enterobacteriaceae raised concerns about their distribution and genetic diversity. Thus, a molecular survey on the detection of *mcr-5*-harbouring *Escherichia coli* was initiated.

In the German national monitoring programme for antimicrobial resistance in zoonotic agents the National Reference Laboratory for Antimicrobial Resistance has received 19216 *E. coli* isolates from food and food-producing animals between 2010 and 2017 for antimicrobial resistance testing. Of these, 737 isolates exhibited an MIC of colistin ≥4 mg/L. During routine laboratory work, all colistin-resistant isolates were subjected to *mcr-1* qPCR screening as previously described.[8] Using the PCR assay of Borowiak *et al.*[7] on *mcr-1*-negative *E. coli* ($n = 135$), three *mcr-5*-positive isolates were detected in samples recovered from the caecal contents of pigs at slaughter (10E01066) and faecal samples from pigs at farms (11E02380, 15-AB00674). These isolates exhibit a non-WT phenotype (resistant) for colistin and other antimicrobials using the microdilution method according to CLSI guidelines (M07-A9) following EUCAST epidemiological cut-off values (Table S1, available as Supplementary data at *JAC* Online).

S1-PFGE profiling and DNA hybridization[9] showed a plasmid location of *mcr-5* in the isolates. Short-read paired-end MiSeq sequencing of genomic DNA and *de novo* assembling were performed as previously described.[7] Relevant genome features of the isolates are summarized in Table S1. The complete genomes of the *mcr-5*-harbouring plasmids were derived from WGS data by raw-read mapping against *Salmonella* Paratyphi B (*d*Ta+) plasmid pSE13-SA01718 (KY807921). In 10E01066 and 11E02380 *mcr-5* was detected on 12201 bp (pEC1066, MG587003) and 11708 bp (pEC2380, MG587004) plasmids, respectively. Comparative analyses using BRIG[10] revealed that pEC1066 and pEC2380 are ColE plasmids with a close relationship to pSE13-SA01718 of *Salmonella* Paratyphi B (*d*Ta+) (Figure 1a).[7] Isolate 15-AB00674 carries *mcr-5* on a 6268 bp plasmid (pEC0674, MF684783) (Figure 1b) exhibiting a stronger similarity to *Klebsiella pneumoniae* plasmid Kp13 (CP003996.1), potentially belonging to an as yet unknown incompatibility type. Interestingly, pEC0674 lacks *tnpA* and *tnpR* of the *mcr-5* transposon.

Further DNA alignments showed that pEC1066 and pEC2380 carry identical *mcr-5* transposon sequences as described for pSE13-SA01718.[7] Interestingly, *mcr-5* on pEC2380 exhibits a deletion of three nucleotides encoding an amino acid in the central part of the protein (Figure 1c). As this isolate exhibits the highest MIC (8 mg/L) of colistin for the isolates of this study (Table S1), this deletion may not affect the domain structure of the enzyme or functional amino acids involved in resistance development (Figure 1c). Since this *mcr-5* allele is the first variant, we suggest designating it *mcr-5.2* (MG384740). The increased colistin resistance (MIC = 8 mg/L) of isolate 11E02380 may be caused by a mutation in *pmrB* (V161G), which is known to contribute to colistin resistance in *E. coli*.[11] In contrast to isolate 11E02380, no colistin resistance-associated *pmrA* or *pmrB* gene mutations were detected in *E. coli* 10E01066 and 15-AB00674.

The *mcr-5*-carrying plasmids of this study do not carry transfer genes involved in plasmid conjugation. Further investigations were performed with pEC1066, which was therefore introduced by transformation into chemically competent *E. coli* cells (DH5α, Thermo Fisher) under colistin (2 mg/L) selection. The presence of



(c)

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pEC1066   MRLSAFITFLKMRPQVRTEFLTLFISLVFTLLCNGVFWNALLAGRDSLTSGTWLMLLCTG   60
pEC2380   MRLSAFITFLKMRPQVRTEFLTLFISLVFTLLCNGVFWNALLAGRDSLTSGTWLMLLCTG   60

pEC1066   LLITGLQWLLLLLVATRWSVKPLLILLAVMTPAAVYFMRNYGVYLDKAMLRNLMETDVRE   120
pEC2380   LLITGLQWLLLLLVATRWSVKPLLILLAVMTPAAVYFMRNYGVYLDKAMLRNLMETDVRE   120

pEC1066   ASELLQWRMLPYLLVAAVSVWWIARVRVLRTGWKQAVMMRSACLAGALAMISMGLWPVMD   180
pEC2380   ASELLQWRMLPYLLVAAVSVWWIARVRVLRTGWKQAVMMRSACLAGALAMISMGLWPVMD   180

pEC1066   VLIPTLRENKPLRYLITPANYVISGIRVLTEQASSSADEAREVVAADAHRGPQ*EQGRRPR   240
pEC2380   VLIPTLRENKPLRYLITPANYVISGIRVLTEQASSSADEAREVVAADAHRGPQ-QGRRPR   239

pEC1066   ALVLVVGETVRAANWGLSGYERQTTPELAARDVINFSDVTSCGTDTATSLPCMFSLNGRR   300
pEC2380   ALVLVVGETVRAANWGLSGYERQTTPELAARDVINFSDVTSCGTDTATSLPCMFSLNGRR   299

pEC1066   DYDERQIRRRESVLHVLNRSDVNILWRDNQSGCKGVCDGLPFENLSSAGHPTLCHGERCL   360
pEC2380   DYDERQIRRRESVLHVLNRSDVNILWRDNQSGCKGVCDGLPFENLSSAGHPTLCHGERCL   359

pEC1066   DEILLEGLAEKITTSRSDMLIVLHMLGNHGPAYFQRYPASYRRWSPTCDTTDLASCSHEA   420
pEC2380   DEILLEGLAEKITTSRSDMLIVLHMLGNHGPAYFQRYPASYRRWSPTCDTTDLASCSHEA   419

pEC1066   LVNTYDNAVLYTDHVLARTIDLLSGIRSHDTALLYVSDHGESLGEKGLYLHGIPYVIAPD   480
pEC2380   LVNTYDNAVLYTDHVLARTIDLLSGIRSHDTALLYVSDHGESLGEKGLYLHGIPYVIAPD   479

pEC1066   EQIKVPMIWWQSSQVYADQACMQTHASRAPVSHDHLFHTLLGMFDVKTAAYTPELDLLAT   540
pEC2380   EQIKVPMIWWQSSQVYADQACMQTHASRAPVSHDHLFHTLLGMFDVKTAAYTPELDLLAT   539

pEC1066   CRKGQPQ   547
pEC2380   CRKGQPQ   546
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Figure 1. Dissection of the *mcr-5*-harbouring plasmids of *E. coli* 10E01066, 11E02380 and 15-AB00674. (a) Organization of *mcr-5*-harbouring plasmids from *E. coli*. The central circle represents the GC content of the reference plasmid. (b) A gene map of the complete nucleotide sequence of plasmid pEC0674 (assemblies from raw reads of isolate 15-AB00674). Relevant features of the plasmid sequences are indicated. (c) An MCR-5 protein comparison (Accelrys DS Gene, version 2.5) of *E. coli* plasmids pEC1066 and pEC2380. Different domains predicted in the primary structure of MCR-5 are indicated as previously described.[7] The domains of transmembrane structures and sulphatases are indicated in dark and light grey, respectively. Sequences highlighted in mid grey illustrate DUF1705 domains. Conserved amino acids that may be involved in substrate binding and colistin resistance are indicated with bold letters. The position of the amino acid deletion is indicated by an asterisk.

pEC1066 in the transformants was confirmed by S1-PFGE and broth microdilution. An MIC of 4 mg/L was observed for DH5α transformants harbouring pEC1066, while cells without this plasmid exhibited an MIC ≤1 mg/L. Conjugation studies in *E. coli* with (F, RP4, RK2) and without helper plasmids indicated that pEC1066 was not transmissible by the mating systems and conditions used (room temperature, 37°C, 42°C).

Our findings indicate that *mcr-5*-harbouring *E. coli* isolates sporadically occur in the intestinal tracts of pigs in Germany, which raises the concern of vertical transmission along the food chain and to consumers. The observed isolates have different characteristics and carry *mcr-5* on two different plasmid types. The resistance gene is located on the Tn*3*-family transposon Tn*6452* on the *Salmonella* Paratyphi B (*d*Ta+) chromosome and

plasmids.[7] To determine the impact of *mcr-5*, further information on the stability of the *mcr-5*-harbouring plasmids, their transmission routes and distribution in livestock, food products and humans is needed. These data are necessary to develop efficient risk management strategies to control the spread of mobile phosphoethanolamine transferase conferring reduced susceptibility to colistin in Enterobacteriaceae.

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Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online.

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Poor palatability of the new ritonavir formulation is a major obstacle to adherence to treatment in young children

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Sir,

Achieving and maintaining good long-term adherence to treatment and viral suppression remains challenging in children perinatally infected with HIV. Overall virological failure (VF) rates in this group are more than double those in adults, even in high-resource countries.[1] Suboptimal virological suppression may lead to the emergence of drug-resistant viruses, the consequences of which are particularly significant for children who will need antiretrovirals