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Location of colistin resistance gene *mcr-1* in Enterobacteriaceae from livestock and meat

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Sir,

The transferable colistin resistance gene, *mcr-1*, was recently described in *Escherichia coli* from livestock, food and humans in China.[1] Within a short period, the gene was also reported in Denmark,[2] rapidly followed by reports in other countries demonstrating a worldwide dissemination of the gene from various sources.[3] In the Netherlands, *mcr-1* was detected at low prevalence in *E. coli* isolates from livestock (<1%) and meat (2%),[3] *Salmonella* from poultry meat (1%; K. Veldman, R. Heymans and W. van Pelt, unpublished data) and rarely in *E. coli* isolated from human patients with a travel history outside Europe.[4] To get a better understanding of the epidemiology of the gene, the *mcr-1*-positive isolates from livestock and meat were molecularly characterized.

The selection consisted of 26 *E. coli* from faecal samples of veal calves ($n=15$), broilers ($n=10$) and turkey ($n=1$) and 13 *Salmonella* obtained from nationally produced broiler meat ($n=11$) and imported turkey meat ($n=2$). All isolates exhibited reduced susceptibility to colistin with MICs between 4 and 16 mg/L (data not shown) and were confirmed as *mcr-1* carriers using conventional PCR according to the EURL-AR protocol (http://www.crl-ar.eu/data/images/protocols/mcr-1_pcr_protocol_v1_dec2015.pdf), followed by Sanger sequencing of the amplicons. Transfer of *mcr-1* was proven effective in broth or filter mating experiments for all *Salmonella* and for most of the *E. coli* (20 out of 26) isolates using a rifampicin-resistant *E. coli* K12 strain as recipient and LB agar containing 100 mg/L rifampicin and 2 mg/L colistin as selective agar. The location of *mcr-1* was determined in the parent strains with S1-PFGE of total DNA followed by Southern blot hybridization using digoxigenin-labelled probes targeting *mcr-1* and two different plasmid replicons: IncHI2 and IncX4. IncHI2 carrying *mcr-1* plasmids (sized: 200–290 kb) were identified in 14 *E. coli* isolates from veal calves ($n=9$) and broilers ($n=5$). The majority of these plasmids were subsequently typed as multireplicon IncHI2/P ($n=12$) with an additional IncP probe. Among the IncHI2 plasmids, ST4 was the predominant double locus ST ($n=11$).[5] The frequent finding of *mcr-1*-positive IncHI2/ST4 plasmids in *E. coli* isolates in our study indicates an important role of this plasmid in the spread of *mcr-1* in livestock as suggested in previous studies.[6,7] Furthermore, a 30 kb IncX4 plasmid was the identified carrier of *mcr-1* in all *Salmonella* isolates ($n=13$) and in 4 *E. coli* isolated from two broilers, one veal calf and one turkey. In two *E. coli* from broilers, two bands with estimated sizes of 20 and 60 kb repeatedly showed hybridization with *mcr-1* and IncX4 probes (data not shown), indicating the presence of two IncX4 plasmid variants carrying *mcr-1*. Remarkably, chromosomal location of *mcr-1* was confirmed in two *E. coli* isolates from veal calves by I-CeuI-PFGE of total DNA and Southern blot hybridization with *mcr-1* and 16S rDNA probes. Because hybridization with *mcr-1* probes failed with Southern blots of S1-PFGE and I-CEUI-PFGE, the location of *mcr-1* remains undefined in four *E. coli* isolates (Table 1).

To screen for the upstream presence of IS*Apl1*, all bacterial isolates were examined with PCR using primers ISApl1-mcr-F (5′–TGGACATTGGGAAGCCGATA–3′) and ISApl1-mcr-R (5′–GCCACAAGAACAAACGGACT–3′), followed by Sanger sequencing of the amplicon. We confirmed IS*Apl1* presence in all 14 *mcr-1*-carrying IncHI2 plasmids and in both *E. coli* isolates with the chromosomally located *mcr-1*. Except for one deletion (position 1073) in the region between IS*Apl1* and *mcr-1*, sequence analysis of the amplicons revealed complete similarity with the IS*Apl1*-mcr element (GenBank accession number KP347127). The presence of IS*Apl1* next to *mcr-1* on the chromosome underlines the significance of this element in the dissemination of the gene. IS*Apl1* was not identified in *mcr-1*-carrying IncX4 plasmids, which is in concordance with earlier findings where IS*Apl1* was identified on IncHI2 and IncI2, but not on IncX4 plasmids.[8] MLST of *E. coli* isolates demonstrated the genetic variety of the isolates with eight different STs per animal species (veal calves and broilers) belonging to at least six clonal complexes (see Table 1). *E. coli* ST10 was found ($n=4$) in both veal calves and broilers. ST648 was found in three veal calves and ST752 was found in three broilers. All other STs were solely found in one animal species.

To the best of our knowledge, we report the first finding of a chromosomally located *mcr-1* gene in two *E. coli* isolates from veal calves. The fact that IS*Apl1* was identified upstream of the *mcr-1* gene strongly suggests that *mcr-1* was able to translocate to the chromosome due to the presence of this IS. Furthermore, our results confirm the implication of IncX4- and IncHI2-type plasmids in the spread of *mcr-1* in Enterobacteriaceae in livestock and meat. To reveal the current spread of *mcr-1* in bacterial populations from different animal sources, prospective screening is urgently needed and should be implemented in existing national surveillance programmes.

Table 1. Characteristics of *mcr-1*-positive *E. coli* ($n=26$) and *Salmonella* ($n=13$) from livestock and meat

Species (*n*)	Location of *mcr-1*	IS*Apl1*	Source	Isolation year	MLST ST(s) (*n*)
E. coli (2)	IncX4 (30 kb)	no	broiler	2011–13	ST1730, ST4512
E. coli (2)	IncX4 (20+60 kb)	no	broiler	2010–13	ST752 (2)
E. coli (1)	IncX4 (30 kb)	no	turkey	2011	ST1564
E. coli (1)	IncX4 (30 kb)	no	veal calf	2010	ST57
Salmonella Java (11)	IncX4 (30 kb)	no	chicken meat	2010–15	NA
Salmonella Anatum (1)	IncX4 (30 kb)	no	turkey meat	2013	NA
Salmonella Schwartzengrund (1)	IncX4 (30 kb)	no	turkey meat	2015	NA
E. coli (1)	IncHI2/ST2 (200 kb)	yes	veal calf	2011	ST410
E. coli (1)	IncHI2/ST4 (275 kb)	yes	veal calf	2010	ST648
E. coli (1)	IncHI2/P/ST3 (290 kb)	yes	broiler	2012	ST10
E. coli (7)	IncHI2/P/ST4 (240–275 kb)	yes	veal calf	2010–11	ST10, ST57, ST624, ST648 (2), ST1011, STnew
E. coli (3)	IncHI2/P/ST4 (225–245 kb)	yes	broiler	2012	ST38, ST752, ST2309
E. coli (1)	IncHI2/P/ST6 (240 kb)	yes	broiler	2012	ST351
E. coli (3)	not defined	yes	veal calf	2011	ST10, ST410, ST648
E. coli (1)	not defined	yes	broiler	2012	ST10
E. coli (2)	chromosomal	yes	veal calf	2010–11	ST57 (2)

NA, not applicable.

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Transparency declarations

None to declare.

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