

1 Diverse *Escherichia coli* lineages, from domestic animals and humans in a household, carry 2 colistin resistance gene *mcr-1* in Ecuador

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15 Running Head: Diverse commensal *E. coli* lineages carrying *mcr-1* gen

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23 **Abstract**

24 The aim of this study was to investigate the presence of *Escherichia coli* carrying *mcr-1* gene
25 in domestic animals close to a child who suffered a peritoneal infection by a *mcr-1* positive
26 *E. coli*. Rectal or cloacal swabs and fecal samples from domestic animals were plated on
27 selective media to isolate colistin-resistant *E. coli* and isolates were submitted to detection of
28 *mcr-1* gene, pulsed field gel electrophoresis (PFGE), multi-locus sequence typing (MLST),
29 replicon typing and S1-PFGE. Four *mcr-1* positive *E. coli* isolates (from chicken, turkey and
30 dog) were recovered. No shared PFGE pattern or MLST sequence type were observed among
31 isolates. A 60Kb IncI1γ *mcr-1*-carrying plasmid was detected in all isolates. Our results
32 suggest that *mcr-1* gene was horizontally disseminated amongst different lineages of *E. coli*
33 from domestic animals in the child's household.

34 **Importance**

35 Horizontally transferable colistin resistance (*mcr-1* gene) is thought to have originated in
36 domestic animals and transferred to humans through meat and dairy products. In the present
37 report we show evidence that the *mcr-1* gene could be transferred to different *E. coli* strains
38 colonizing different hosts (humans and pets) in the same household.

39 **Introduction**

40 Domestic animals are important source of antibiotic resistant bacteria (and genes) which can be
41 transmitted to humans through the food chain and direct contact (1). Plasmids and other mobile
42 genetic elements (MGEs) are involved in the transmission of antimicrobial resistance among
43 different bacterial genera colonizing different animal species (2, 3).

44 The first report of a colistin resistance (CR) gene carried by plasmids (*mcr-1*) came from China in
45 2015 (4). This gene codes for a phosphoethanolamine transferase (MCR-1), which modifies the
46 lipid A moiety in the outer membrane of Gram negative bacteria and confers resistance to
47 polymyxins (4, 5). Among Enterobacteriaceae different *mcr* gen groups (1-5) could be transferred by
48 mobile elements (6-8) and have been detected in humans, food animals, environmental samples in
49 several countries, in different bacterial species and in several plasmid types (9). Enterobacteriaceae
50 has been found as the main *mcr* reservoir (6-8). The gene is easily transferred between commensal
51 *E. coli* colonizing domestic animals and humans (7, 10) and opportunistic pathogens such as *E.*
52 *coli* ST3941 (11) or *Klebsiella pneumoniae* ST512 KPC-3 (7, 12, 13).

53

54 In Ecuador, Ortega *et al.* have described the isolation of a colistin resistant *E. coli* carrying *mcr-1*
55 from the peritoneal liquid in a child with complicated peritonitis (14). We investigated the *mcr-1*
56 gene in *E. coli* isolates from domestic animals in this child's household.

57 **Results**

58 Four colistin resistant *E. coli* isolates were recovered from a dog (2 strains), a turkey (1 strain) and
59 a chicken (1 strain). Susceptibility test for each strain is shown in **Table 1**. All CR isolates (colistin
60 (MIC >4 µg/mL) were also resistant to ceftriaxone (MIC ≥64 µg/mL) and ciprofloxacin (MIC ≥4
61 µg/mL). Additionally, dog isolates were resistant to ampicillin/sulbactam (MIC ≥32 µg/mL) and one
62 of their isolates were resistant to gentamicin (MIC ≥16 µg/mL).

63 Sequenced PCR products showed to be identical among them and close related to *mcr-1* sequences
64 that were previously reported (NCBI Accession number: KX11520.1, KX011521, KU935449.1,
65 KU935446.1, KU935447, NG050417, KY013597).

66 Both PFGE and MLST indicated that CR *E. coli* isolates from domestic animals and the child (14)
67 were different (Figure 1, Table 1). Sequence types (STs) carrying the *mcr-1* gene in the patient was
68 ST609 and in the animal samples ST3941, 2170 and 1630. The isolates recovered from chicken and
69 turkey also harbored *bla*CTX-M-65 (the one from turkey was *bla*TEM-1 as well), and the 2 *E. coli* from
70 the dog were positive for *bla*CTX-M-3 and *bla*TEM-1 genes. All CR isolates from domestic animals in
71 the household and the isolate from the child had an IncI1γ plasmid (Table 1). We were unable to
72 transfer the *mcr-1* gene using the conditions described in methods section. S1-PFGE showed a ≈
73 60Kb plasmid positive for *mcr-1* gene in all isolates (from animal and human) (Figure 1).

74 **Discussion**

75 *E. coli* genetic diversity has been studied in different environments showing no clear association
76 patterns among different sources (15). In this study, colistin resistant *E. coli* strains isolated from
77 different sources belonged to different lineages and no one was comparable with child isolate despite
78 of they have an identical sequence.

79 *E. coli* ST 609 (child strain) has been previously detected in human (commensal and opportunistic
80 pathogens), and water isolates in Spain, Argentina, United States, Vietnam, Netherlands, Australia,
81 and Norway. ST 3941(chicken strain) has been identified in commensal bacteria from livestock and
82 poultry in China and Vietnam farms, and *E. coli* ST 2170 (turkey strain) has been reported as an
83 opportunistic pathogen causing septicemia in Japan and in healthy poultry farms in Vietnam. ST 1630
84 (Dog strains) was isolated from chickens in Denmark and United States (reported data in
85 <http://mlst.ucc.ie/mlst/dbs/Ecoli>; last accessed December 8, 2017). Moreover, ST 1630 was also
86 isolated from poultry in Japan (16). These data show the diverse and spread sources of *E. coli* strains
87 detected and support the idea that successful mobile genetic elements could be responsible of

88 antimicrobial resistance transference among dominant strains of commensal bacteria in different
89 niches.

90 We were unable to transfer the *mcr-1* gene to an acceptor, sodium azide resistant *E. coli*. Previous
91 reports have described a low transfer frequency of *mcr-1*-carrying plasmids that could depends of
92 plasmid type (16). However, we cannot discard that the conditions used in our study were not the
93 optimal for their horizontal transfer.

94 Using a specific *mcr-1* probe, one of the isolates (from turkey) showed two positive bands by
95 Southern blot analysis: the common plasmid (~61 kb) and another one at ~140 kb. Li R *et al*
96 (2016), have reported double plasmid carrying *mcr-1* gene in commensal *E. coli* strains isolated
97 from healthy pigs in China farms (17, 18). The genetic analysis of those plasmids showed a
98 composite transposon Tn6330 (2 copies of ISApII flanking the *mcr-1* gene) that can form a circular
99 intermediate which mediates the insertion of the *mcr-1* gene cassette into the IncHI2 plasmid.
100 Further analysis is required to describe the *mcr-1* genetic environment and complete structure of
101 plasmids from *E. coli* strains isolated in our study.

102 Both S1-PGFE and replicon typing suggest that the *mcr-1* gene was present in the same plasmid in
103 all CR isolates (from domestic animals and human). It is probable that \approx 60Kb plasmids were
104 responsible for the transference of colistin resistance; previous reports have shown transmission of
105 antibiotic resistance from bacteria in domestic animals to human bacteria is carried out by plasmids
106 and not by antibiotic resistant clones (3).

107 Our study corroborates the notion that Enterobacteriaceae colonizing intestines in domestic animals
108 (including companion animals) could be reservoir of *mcr-1* gene (2, 19–22) . There was no
109 evidence of colistin treatment in animals sampled, however, the presence of this type of antibiotic

110 in animal feed and supplements in Ecuador prevents us from rule out antibiotic selection of CR
111 clones.

112 **Materials and Methods**

113 *2.1 E. coli strain isolation and MIC determination*

114 A cross – sectional study was conducted to detect commensal *E. coli* carrying *mcr-1* gene. Thirty-
115 two fecal samples from soil and ten rectal or cloacal swabs were taken from rabbits (n= 2), guinea
116 pigs (n= 2), dogs (n= 2) and chickens (n= 4). Fecal samples (n=32) from soil were placed in sterile
117 reservoirs and swabs were placed in Tryptic Soy Broth (TSB, BDTM) (19). Samples were
118 transported to the Antimicrobial Resistance Laboratory in the Instituto Nacional de Investigación en
119 Salud Pública "Dr. Leopoldo Izquieta Perez", Quito. Samples were plated on Mac Conkey Agar
120 plates (MKL, BDTM) supplemented with 2 µg/mL of colistin methansulfonate (RICHET®) (20).
121 Identification and antimicrobial susceptibility profiles of the CR isolates were performed using the
122 VITEK®2 compact (bioMérieux) with AST 272 card. Colistin minimal inhibitory concentration
123 (MIC) was performed using SensititreTM (23).

124 *2.2 Conjugation assay*

125 Conjugation was performed using CR *E. coli* isolates as donor strains and *E. coli* J53 strain (sodium
126 azide-resistant) as recipient (17). Trans-conjugant selection was performed in Trypticase TM soy
127 agar (Difco BD) with colistin (0.5µg/mL) and sodium azide (100 µg/mL) (24).

128

129 *2.3 Molecular typing*

130 PCR was performed to detect *mcr* gene (4); amplicons were sequence and aligned using *mcr-1*
131 (*NG_055582.1*), *mcr-2* (*NG_051171.1*), *mcr-3* (*NG_056184.1*), *mcr-4* (*MG822665.1*), *mcr-5*
132 (*MG384740.1*) accession numbers with Geneius software. Pulsed field gel electrophoresis (PFGE)
133 (25) and multilocus sequence typing (MLST) was performed on seven housekeeping genes to
134 define clonal relatedness (26). Replicon typing was performed using a commercial kit (PBRT KIT,
135 DIATHE, Fano, Italy) (20, 27, 28). β -lactamase genes (*blaCTX-M-1*, *blaTEM*, *blaSHV*) were detected
136 using primers previously described (29). Briefly, 12,5mL of GoTaq® Green Master Mix
137 (Promega, Madison, USA) were mixed with 1 μ L of upstream primer, 10 μ M, 1 μ L of downstream
138 primer, 10 μ M, 1 μ L of DNA template and 9,5 μ L of Nuclease-free water to complete a 25 μ L of
139 reaction volume. The reaction mix were amplified using 2-minute of initial denaturation at 94°C
140 followed by 40 cycles of DNA denaturation at 94°C (40 sec), annealing at 60°C (40sec) and
141 extension at 72°C (1min). The final elongation step at 72°C for 5 min. Amplicons were detected by
142 electrophoresis in a 2% agarose gel. For complete amplification and sequencing of the detected
143 resistance genes, primers and conditions previously described were used (30).

144 *S1-PFGE and Southern blot.*

145 Plasmid content of each *E. coli* isolate and their estimated sizes were determined by S1
146 endonuclease-digested genomic DNA and PFGE (S1-PFGE). Briefly, genomic DNA agarose plugs
147 of each isolate were partially digested with the endonuclease S1(31). DNA bands were separated by
148 PFGE under previously described conditions (32). Plasmids were transferred and immobilized on a
149 nylon membrane and identified by Southern blot analysis, using specific *mcr-1* digoxigenin-labeled
150 probes (Roche Diagnostics).

151

152 **Conclusions**

153 Our study suggests a polyclonal dissemination of *mcr-1* gene in *E. coli* domestic animals and
154 humans in an Ecuadorian household. A 60Kb IncI1γ plasmid carried the *mcr-1* gene in this study,
155 which may have been transferred among different strains colonizing different hosts. This study
156 supports the current notion that mobile gene elements are more important than bacterial clones in
157 the transmission of antibiotic resistance genes from the microbiota in domestic animals to human
158 microbiota (33).

159 Our results highlight the importance of controlling the use of antibiotics in domestic animals and
160 possibly the need for more studies of AR in isolates and mobile genetic elements in them.

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166 **Competing interest:** The authors declare that they don't have any conflict of interests.

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277 **Table 1.** Antibiotic susceptibility profiles and molecular analyses (PCR for *bla* genes detection, MLST and Replicon typing) of *mcr-1*-positive *E. coli*
 278 isolates.

Strain origin	Antimicrobial resistance	MLST		PCR		Plasmid Inc group								
		ST	<i>bla</i> genes	HI1	I2	N	FIA	FIB	I1γ	FIIS	R	X1	Y	FII
Chicken	COL, CRO, CIP	3941	<i>bla</i> _{CTX-M-65}	-	+	-	+	-	+	-	-	-	-	-
Turkey	COL, CRO, CIP	1630	<i>bla</i> _{TEM-1} ; <i>bla</i> _{CTX-M-65}	+	-	-	-	-	+	-	-	-	-	-
Dog1	COL, SAM, CRO, CIP	2170	<i>bla</i> _{TEM-1} ; <i>bla</i> _{CTX-M-3}	-	+	-	-	+	+	+	-	-	+	+
Dog2	COL, SAM, CRO, CIP, GN	2170	<i>bla</i> _{TEM-1} ; <i>bla</i> _{CTX-M-3}	-	-	-	-	-	+	-	-	-	-	-
Child*	COL, CAZ, CRO, FEP, CIP	609 [13]	<i>bla</i> _{CTX-M-55} [13]	-	+	+	-	-	+	-	+	+	-	-

279 COL, colistin; SAM, ampicillin/sulbactam; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; GN, gentamicin; CIP, ciprofloxacin.

280 ST, sequence type obtained by MLST analysis; *bla* genes, β-lactamase genes; Poultry TC, Transconjugant strain obtained with conjugation using
 281 chicken CR *E. coli* isolate as donator and *E. coli* J53 as receptor strain. Replicon typing analysis was performed with PBRT KIT (DIATHEVA):
 282 only positive Inc groups in the overall analysis are included. * isolate reported by Ortega *et al*

283

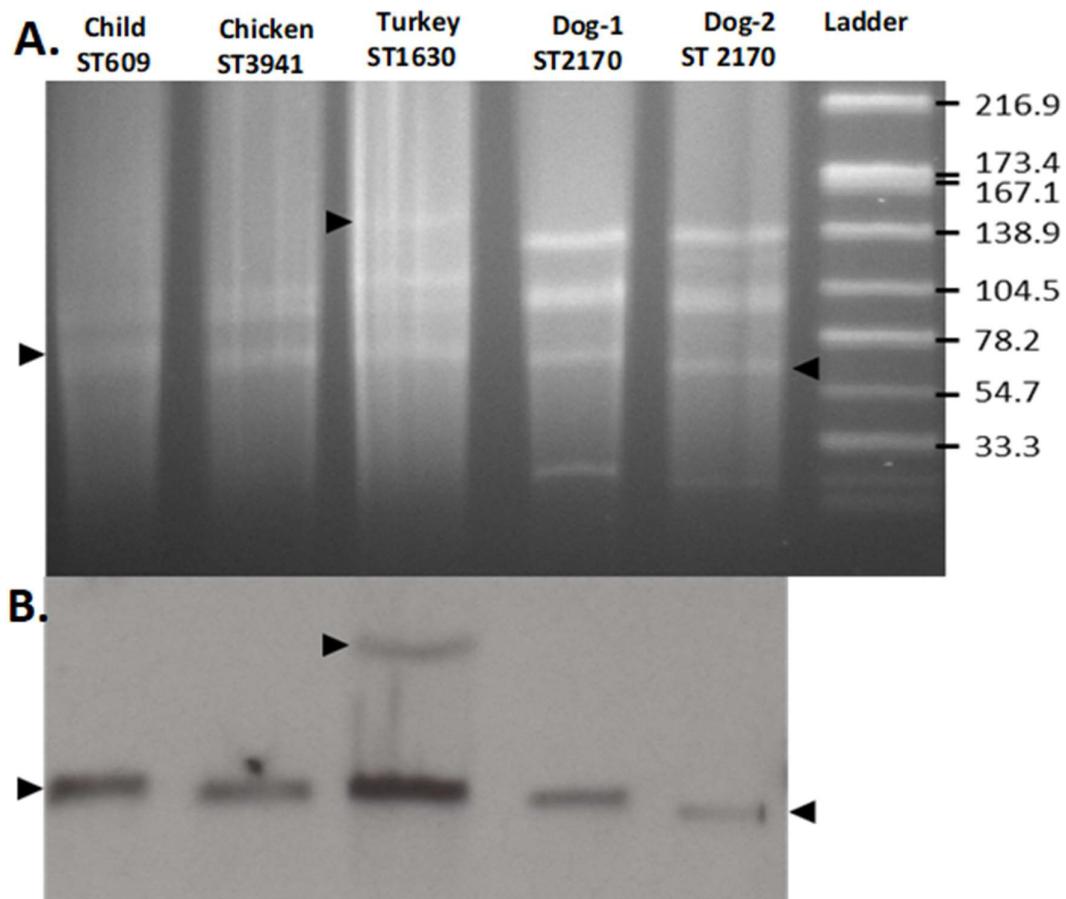


Figure 1. Identification of *mcr-1*-harboring plasmids. (A) S1- PFGE plasmid profiles of the patient and animals *mcr-1* positive *E. coli*. (B) Southern blot using *mcr-1* probe. Arrows indicate the plasmids carrying *mcr-1* gene. Ladder, reference standard *Salmonella enterica* serotype Braenderup strain H9812 restricted with *Xba*I (sizes are given in kilobases)