Silent dissemination of colistin-resistant *Escherichia coli* in South America could contribute to the global spread of the *mcr-1* gene

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During a Brazilian multicentric antimicrobial resistance surveillance study, colistin resistance was investigated in *4,620 Enterobacteriaceae* isolated from human, animal, food and environmental samples collected from 2000 to 2016. We present evidence that *mcr-1*-positive *Escherichia coli* has been emerging in South America since at least 2012, supporting a previous report on the possible acquisition of *mcr-1*-harbouring *E. coli* by European travellers visiting Latin American countries.

We present evidence that *mcr-1*-harbouring *Escherichia coli* has been occurring in food-producing animals in Brazil since at least 2012.

**Screening Enterobacteriaceae isolates for potential colistin resistance and the *mcr-1* gene**

Between 2000 and 2016, a total of *4,620 Enterobacteriaceae* isolates were collected in Brazil, as part of different surveillance projects on carbapenemase- and/or extended-spectrum beta-lactamases (ESBL)-producing Gram-negative bacteria important to human and veterinary medicine [1-4]. Within this Brazilian multicentric antimicrobial resistance surveillance study, we hereby also investigate colistin resistance.

The *4,620 isolates* were screened using MacConkey agar plates supplemented with colistin (2 mg/L). A total of *515 isolates*, which had grown on the screening plates were obtained. These originated from food-producing animals (227 isolates), chicken feed (4 isolates), companion (9 isolates) and non-companion animals (24 isolates), humans (137 isolates), food (102 isolates) and the environment (12 isolates). The *515 isolates* were further tested for susceptibility to colistin by agar dilution and/or broth microdilution method, whereby a minimum inhibitory concentration (MIC) > 2 mg/L was considered indicative of colistin resistance according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [5]. Isolates were also subjected to polymerase chain reaction (PCR) to check whether respective strains harboured the *mcr-1* gene [6], which if present was sequenced (Table).

The *mcr-1* gene was detected in 16 commensal *E. coli* strains exhibiting colistin MICs from 1 to 16 mg/L (MIC<sub>90</sub> = 8 mg/L). Two of the *mcr-1*-positive *E. coli* strains were found in faecal samples collected in 2012 from healthy pigs in farms located in Santa Catarina and Minas Gerais states. One of these two isolates was susceptible for colistin (MIC = 1 mg/L). The remaining 14 *mcr-1*-harbouring *E. coli* strains originated from faecal samples of healthy chickens, which had been gathered in 2013 from farms located in Paraná, São Paulo and Minas Gerais states. All 14 isolates from chickens had a MIC ≥ 8 mg/L.

**Relationships between *mcr-1*-positive isolates, and testing for extended-spectrum beta-lactamases**

The sequences of the 16 *mcr-1*-positive *E. coli* strains were phylogenetically analysed [7], revealing that 11
**Figure 1**
Pulsed-field gel electrophoresis (PFGE) and antimicrobial resistance characteristics of mcr-1-positive *Escherichia coli* strains isolated from faeces of healthy livestock, Brazil, 2012–2013

### A. *XbaI* PFGE of MCR-1-positive *E. coli* strains isolated from faeces of healthy livestock

![PFGE Pattern](image.png)

### B. Relationship between isolates obtained after *XbaI* PFGE and antimicrobial resistance

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Source</th>
<th>Year</th>
<th>State</th>
<th>Resistance profile (Kirby–Bauer)</th>
<th>Colistin MIC (mg/L)</th>
<th>β-lactamase</th>
<th>Phylgroup</th>
<th>PFGE cluster</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.3</td>
<td>Chicken</td>
<td>2013</td>
<td>PR</td>
<td>CRO, CTX, CTF, CPM, TET</td>
<td>8</td>
<td>CTX-M-1</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>662</td>
<td>Chicken</td>
<td>2013</td>
<td>SP</td>
<td>–</td>
<td>8</td>
<td>–</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>12.3</td>
<td>Chicken</td>
<td>2013</td>
<td>PR</td>
<td>CRO, CTX, TET</td>
<td>16</td>
<td>CTX-M-8</td>
<td>A</td>
<td>C</td>
</tr>
<tr>
<td>9.6</td>
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<td>PR</td>
<td>CRO, CTX</td>
<td>8</td>
<td>CTX-M-8</td>
<td>A</td>
<td>C</td>
</tr>
<tr>
<td>2.6</td>
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<td>PR</td>
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<td>CTX-M-8</td>
<td>A</td>
<td>C</td>
</tr>
<tr>
<td>3.6</td>
<td>Chicken</td>
<td>2013</td>
<td>TET</td>
<td></td>
<td>8</td>
<td>–</td>
<td>B1</td>
<td>E</td>
</tr>
<tr>
<td>5.5</td>
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<td>PR</td>
<td>CRO, CTX, CTF, CPM, TET</td>
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<td>CTX-M-15</td>
<td>A</td>
<td>F</td>
</tr>
<tr>
<td>146</td>
<td>Swine</td>
<td>2012</td>
<td>SC</td>
<td>AMC, FOX, CLO, SXT, TET</td>
<td>1</td>
<td>CMY-2</td>
<td>A</td>
<td>G</td>
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<tr>
<td>11.3</td>
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<td>PR</td>
<td>CRO, CTX, CTF, CPM, CIP, ENO</td>
<td>8</td>
<td>CTX-M-8</td>
<td>B1</td>
<td>H</td>
</tr>
<tr>
<td>11.8</td>
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<td>PR</td>
<td>CRO, CTX, CTF, CPM, CIP, ENO</td>
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<td>CTX-M-8</td>
<td>B1</td>
<td>H</td>
</tr>
<tr>
<td>9.3</td>
<td>Chicken</td>
<td>2013</td>
<td>PR</td>
<td>CRO, CTX, CPM, CIP, ENO</td>
<td>8</td>
<td>CTX-M-8</td>
<td>B1</td>
<td>H</td>
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<tr>
<td>96</td>
<td>Chicken</td>
<td>2013</td>
<td>MG</td>
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<td>8</td>
<td>–</td>
<td>A</td>
<td>I</td>
</tr>
<tr>
<td>5.2</td>
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<td>ENO</td>
<td>8</td>
<td>–</td>
<td>A</td>
<td>J</td>
</tr>
<tr>
<td>171</td>
<td>Swine</td>
<td>2012</td>
<td>MG</td>
<td>CLO, SXT</td>
<td>8</td>
<td>–</td>
<td>B1</td>
<td>nt</td>
</tr>
</tbody>
</table>

**MIC**: minimum inhibitory concentration; nt: non typeable by PFGE.

GenBank accession number for mcr-1 genes identified in this study: KU750813, KU928239–42, KU935441–9, KX03152–1.

a The marker (M) used was the Lambda ladder 0.05–1Mb, Bio-Rad. Separation of fragments was carried out at 6V/cm at 14°C for 20h, with linear pulse time of 3.51s to 30.82s.

b The states were as follow: MG: Minas Gerais state (South-east Brazil); PR: Paraná state (South); SC: Santa Catarina state (South); SP: São Paulo (South-east).

c The antimicrobial susceptibility was evaluated by disc diffusion assay. Extended-spectrum beta-lactamase (ESBL) production was investigated by using a double-disc synergy test (DDST) [23,24]. AMC: amoxicillin/clavulanic acid; CAZ: ceftazidine; CFX: cefotaxime; CIP: ciprofloxacin; CLO: chloramphenicol; CPM: cefepime; CRO: ceftiraxone; CTX: cefotaxime; ENO: enrofloxacin; FOS: fosfomycin; GEN: gentamicin; SXT: trimethoprim/sulfamethoxazole; TET: tetracycline.

d MICs were determined according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [5,25]. Colistin resistance was defined as a colistin MIC ≥ 2 mg/L, according to EUCAST clinical breakpoints [5].

e PFGE patterns were analysed using the Dice similarity with coefficient optimisation set at 1% and tolerance at 2% (BioNumerics software; Applied Maths, Kortrijk, Belgium).
Figure 2
Geographical distribution of mcr-1-positive *Escherichia coli* isolates reported from South America, 2012–2016

A light grey colour is used for Brazil, where this study was conducted. The dark grey colour indicates countries (Bolivia, Colombia and Peru) visited between November 2012 and November 2013, by unrelated Dutch travellers, for whom acquisition of faecal colonisation and carriage with MCR-1 and extended-spectrum beta-lactamase (ESBL)-producing *E. coli* was shown one to two weeks after their return to the Netherlands [12]. A dark grey colour is used for Ecuador, where subsequent to the identification of a human mcr-1-positive isolate, a sequence was deposited in GenBank in March 2016 (GenBank accession number: KU886144.1).
strains belonged to the phylogroup A and five to the phylogroup B1. Clonal relatedness of the strains were further determined by XbaI pulsed-field gel electrophoresis (PFGE) (www.cdc.gov/pulsenet/). PFGE differentiated mcr-1-positive E. coli isolates into 10 distinct pulsotypes (named A to J), which clustered into two major groups, C (n = 4) and H (n = 3) (Figure 1).

Most (n = 9) mcr-1-positive isolates exhibited resistance to human and/or veterinary cephalosporins. In this regard, such isolates harboured bla_{CTX-M-1}, bla_{CTX-M-8} and/or bla_{CTX-M-15} ESBL genes, and one isolate carried the pAmpC bla_{CMY-2} gene. On the other hand, all isolates carrying the mcr-1 gene belonged to low-virulence E. coli phylogroups (i.e. A and B1 as described above).

**Discussion**

The plasmid-mediated colistin (polymyxin E) resistance mechanism MCR-1 was first described in Enterobacteriaceae isolated from animals, food and human beings in China [6]. Since, and as summarised by Skov and Monnet [8], MCR-1 has also been reported to occur in other countries in Asia, Europe and North America. Recent descriptions from Egypt [9], Italy [10]...
and Spain [11] further denote dissemination of the mechanism, while identifications of \textit{mcr-1} positive strains in imported food, urban rivers and travellers [12-16] highlight the potential for MCR-1 to continue spreading. In addition, co-production of ESBLs or carbapenemases by \textit{mcr-1}-harbouring Enterobacteriaceae has now been documented [12,13,15-18].

We report \textit{mcr-1}-positive \textit{E. coli} isolates from food-producing animals in the southern (Santa Catarina and Paraná states) and south-eastern (São Paulo and Minas Gerais states) regions of Brazil (Figure 2). Interestingly, in most of these isolates (9 of 16), \textit{E. coli} strains co-produced CTX-M-type ESBLs.

Our findings moreover suggest that \textit{mcr-1}-harbouring \textit{E. coli} strains have been present in South America since at least 2012, supporting the results of a previous study on the possible acquisition of \textit{mcr-1}-carrying \textit{E. coli} by European travellers visiting this continent (Figure 2) [12]. In this previous prospective study, the carriage of multiresistant bacteria after travel (COMBAT) consortium had shown that unrelated Dutch travellers to Bolivia, Colombia and Peru between November 2012 and November 2013 had become carriers of colonised with MCR-1 and ESBL-producing \textit{E. coli} one to two weeks after their return to the Netherlands [12].

Recently the \textit{mcr-1} gene has also been identified in another Latin American country, Ecuador, whereby a respective sequence from a human clinical \textit{E. coli} isolate was submitted to GenBank (GenBank accession number: KU886144.1) in March 2016. Therefore, hospital laboratories worldwide should be aware of the possibility of MCR-1 in Enterobacteriaceae isolates resistant to polymyxins from patients living in or returning from Latin American countries.

That \textit{E. coli} with plasmid-mediated MCR-1 are found in Brazil is also relevant for medical centres in this country, where the emergence and dissemination of multidrug-resistant pathogens, which is associated with high rates of treatment failure, have led to high use of polymyxins, mainly in intensive care units [19]. There, this class of antimicrobial agents represents the main therapeutic option for treating severe ‘superbug’ infections, particularly \textit{Pseudomonas aeruginosa}, \textit{Acinetobacter baumannii} and \textit{Klebsiella pneumoniae} producing SPM-1, OXA-23 or KPC-2 carbapenemases, which are highly prevalent in most Brazilian hospitals [19]. On a positive note however, our study did not find \textit{mcr-1}-positivity in any of the human isolates screened, which is consistent with the very low background carriage of MCR-1 in humans, as described previously [6,12-14].

Our result that the \textit{mcr-1} gene occurs in Brazilian livestock is a cause for concern in terms of the global contribution of Brazil to national and international movement of people and products, as this could contribute to the acceleration of the worldwide spread of the \textit{mcr-1} gene. Indeed, with a population of 205 million inhabitants, Brazil has continental proportions and is the biggest country in Latin America. Furthermore, in the agribusiness it is the third producer of chicken meat (only after the United States and China) and the largest exporter of this product [20]. In this regard, colistin sulphate is widely used in animal feed as a growth promoter in Brazilian livestock, mainly in pigs and poultry, supporting a link between the agricultural use of colistin and colistin resistance [21].

Finally, the identification of a colistin-susceptible \textit{E. coli} strain carrying the \textit{mcr-1} gene, in this study, suggests that \textit{mcr-1}-positive isolates may be difficult to detect if the \textit{mcr-1} gene is only tested for in colistin resistant isolates. This may contribute to the silent dissemination of \textit{mcr-1} harbouring strains. In fact, many MCR-1 producers are known to exhibit low level of resistance to colistin (i.e. 4–16 mg/L) [6,8,14,16,22].

In summary, since MCR-1-producing strains have already become established in South America, we emphasise the need for continuous local surveillance programmes to identify the risk to human health. To reduce this risk, the authors suggest that colistin should only be used for treatment of clinical infectious diseases and no longer for animal production, in order to prevent the wide spread of MCR-1-producing bacteria, achieving the principles of responsible use of antibiotics.

\textbf{Erratum}

The term ‘\textit{mcr-1}’ had been mistyped as ‘\textit{mcr-1}’ on several occasions and this was corrected on 02 May 2016.

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\textbf{Conflict of interest}

None declared.

\textbf{Authors’ contributions}

MRF, QM, LS, FE, RL, LKO, DDG, MD, MHM, DFMM, ML, DdOG, TK and AMM collected the data and samples, MRF, QM, LS, KCS, MPVC, FE, RL, MD, GFR, MFCB and NL performed the microbiological and molecular analysis, MRF, QM, KCS, FE, MD, DdOG, TK and NL participated in drafting the manuscript, NL coordinated and edited the manuscript.

\textbf{References}


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