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 (MICp90 = 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 = 1mg/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

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

| Isolate | Source | Year | State | Resistance profile (Kirby – Bauer) | Colistin MIC (mg/L) | β-lactamase | Phylgroup | PFGE cluster |
|---------|--------|------|-------|-----------------------------------|---------------------|-------------|-----------|-------------|
| 5.3     | Chicken | 2013 | PR    | CRO, CTX, CTF, CPM, TET           | 8                   | CTX-M-1     | A         | A           |
| 662     | Chicken | 2013 | SP    | –                                 | 8                   | –           | A         | B           |
| 12.3    | Chicken | 2013 | PR    | CRO, CTX, TET                     | 16                  | CTX-M-8     | A         | C           |
| 12.6    | Chicken | 2013 | PR    | –                                 | 8                   | –           | A         | C           |
| 6.3     | Chicken | 2013 | PR    | CRO, CTX                          | 8                   | CTX-M-8     | A         | C           |
| 9.6     | Chicken | 2013 | PR    | CRO, CTX, CPM, ENO                | 8                   | CTX-M-8     | A         | C           |
| 2.6     | Chicken | 2013 | PR    | CRO, CTX, TET                     | 8                   | CTX-M-15    | A         | D           |
| 3.6     | Chicken | 2013 | PR    | TET                               | 8                   | –           | B1        | E           |
| 5.5     | Chicken | 2013 | PR    | CRO, CTX, CPM, TET                | 8                   | CTX-M-15    | A         | F           |
| 146     | Swine   | 2012 | SC    | AMC, FOX, CLO, SXT, TET           | 1                   | CMY-2       | A         | G           |
| 11.3    | Chicken | 2013 | PR    | CRO, CTX, CPM, CIP, ENO           | 8                   | CTX-M-8     | B1        | H           |
| 11.8    | Chicken | 2013 | PR    | CRO, CTX, CPM, TET, CIP, ENO      | 8                   | CTX-M-8     | B1        | H           |
| 9.3     | Chicken | 2013 | PR    | CRO, CTX, CPM, CIP, ENO           | 8                   | CTX-M-8     | B1        | H           |
| 96      | Chicken | 2013 | MG    | ENO, CIP                          | 8                   | –           | A         | I           |
| 5.2.1   | Chicken | 2013 | PR    | ENO                               | 8                   | –           | A         | J           |
| 171     | Swine   | 2012 | MG    | CLO, SXT                          | 8                   | –           | B1        | nt          |

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, KX01152–1.

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) [5,23,24]. AMC: amoxicillin/clavulanic acid; CAZ: cefazidime; CFX: cefotaxime; CIP: ciprofloxacin; CLO: chloramphenicol; CPM: cefepime; CRO: ceftriaxone; CTI: cefotriaxone; 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).
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 \textit{XbaI} pulsed-field gel electrophoresis (PFGE) (www.cdc.gov/pulsenet/). PFGE differentiated \textit{mcr-1}-positive \textit{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).

The 16 \textit{mcr-1}-positive isolates were additionally tested for the production of extended-spectrum beta-lactamases (ESBLs) by using a double-disc synergy test (DDST) as well as for the presence of ESBL- and plasmid-mediated AmpC (pAmpC) beta-lactamase genes [1,6]. Most (n= 9) \textit{mcr-1}-positive isolates exhibited resistance to human and/or veterinary cephalosporins. In this regard, such isolates harbour \textit{bla}_{CTX-M-1}, \textit{bla}_{CTX-M-8} and/or \textit{bla}_{CTX-M-15} ESBL genes, and one isolate carried the pAmpC \textit{bla}_{CMY-2} gene. On the other hand, all isolates carrying the \textit{mcr-1} gene belonged to low-virulence \textit{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]

### Table

Results of screening \textit{Enterobacteriaceae} isolates from different sources by culture with colistin and presence of the \textit{mcr-1} gene in the screened isolates, Brazil, 2000–2016 (n = 4,620 isolates screened)

| Source\(^a\) | Years of isolate collection | Enterobacteriaceae isolates tested \(n\) | Enterobacteriaceae isolates with growth on screening plates (2 mg/L colistin) \(n^b\) | \textit{Isolates positive for \textit{mcr-1} N (% of isolates screened)}\(^c\) |
| --- | --- | --- | --- | --- |
| Food-producing animals | Chicken | 2003–2015 | 280 | 113 | 14 (5.0) |
| | Swine | 2012–2014 | 113 | 79 | 2 (1.8) |
| | Cattle | 2014–2015 | 158 | 22 | 0 (0) |
| | Goat | 2013 | 7 | 1 | 0 (0) |
| | Ostriches | 2015 | 9 | 2 | 0 (0) |
| | Buffalo | 2010 | 36 | 10 | 0 (0) |
| Chicken feed | – | 2000–2014 | 8 | 4 | 0 (0) |
| Companion animals | Cats | 2013 | 4 | 0 | 0 (0) |
| | Dogs | 2013 | 51 | 9 | 0 (0) |
| Non-Companion animals | Horse | 2013 | 13 | 3 | 0 (0) |
| | Rodents | 2013–2014 | 14 | 13 | 0 (0) |
| | Turtle | 2015 | 21 | 8 | 0 (0) |
| | Urban pigeons | 2015–2016 | 36 | 0 | 0 (0) |
| | Urban waterfowl | 2012–2014 | 75 | 0 | 0 (0) |
| Human infection/colonisation | – | 2004–2016 | 3,591 | 137 | 0 (0) |
| Food | Chicken meat | 2013 | 42 | 22 | 0 (0) |
| | Swine meat | 2012–2014 | 113 | 79 | 0 (0) |
| | Cabbage | 2016 | 2 | 0 | 0 (0) |
| | Lettuce | 2016 | 2 | 0 | 0 (0) |
| | Spinach | 2016 | 1 | 1 | 0 (0) |
| Environment | Lake | 2012–2013 | 20 | 2 | 0 (0) |
| | River | 2011 | 3 | 3 | 0 (0) |
| | Sewage | 2009–2013 | 21 | 7 | 0 (0) |
| Total | – | – | 4,620 | 515 | 16 (0.3) |

\(^a\) Isolates originated from previous surveillance studies of carbapenemase- and/or extended-spectrum beta-lactamases (ESBL)-producing Gram-negative bacteria in food, food-producing animals (faecal samples from healthy animals), chicken feed, companion and non-companion animals (faecal samples from healthy animals), environment and human patients from healthcare settings (27 faecal samples from colonised individuals and 3,564 clinical cultures from infections), all collected in Brazil between 2000 and 2016 [1-4].

\(^b\) Isolates were screened for potential colistin resistance using MacConkey agar plates supplemented with colistin (2 mg/L).

\(^c\) Enterobacteriaceae isolates with growth on screening plates were subjected to \textit{mcr-1} polymerase chain reaction and sequencing [6].
and Spain [11] further denote dissemination of the mechanism, while identifications of 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 mcr-1-harbouring Enterobacteriaceae has now been documented [12,13,15-18].

We report mcr-1-positive 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), E. coli strains co-produced CTX-M-type ESBLs.

Our findings moreover suggest that mcr-1-harbouring 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 mcr-1-carrying 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 E. coli one to two weeks after their return to the Netherlands [12].

Recently the mcr-1 gene has also been identified in another Latin American country, Ecuador, whereby a respective sequence from a human clinical 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 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 Pseudomonas aeruginosa, Acinetobacter baumannii and 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 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 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 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 E. coli strain carrying the mcr-1 gene, in this study, suggests that mcr-1-positive isolates may be difficult to detect if the mcr-1 gene is only tested for in colistin resistant isolates. This may contribute to the silent dissemination of 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.

Erratum

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

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Conflict of interest

None declared.

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, GRF, 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.

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