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 (MIC₅₀ = 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. *Xba*I PFGE of MCR-1-positive *E. coli* strains isolated from faeces of healthy livestock

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

	Isolate	Source	Year	State ^b	Resistance profile (Kirby – Bauer) ^c	Colistin MIC (mg/L) ^d	β-lactamase	Phylogroup	PFGE cluster ^e
70	5.3	Chicken	2013	PR	CRO, CTX, CTF, CPM, TET	8	CTX-M-1	A	A
60	662	Chicken	2013	SP	–	8	–	A	B
50	12.3	Chicken	2013	PR	CRO, CTX, TET	16	CTX-M-8	A	C
40	12.6	Chicken	2013	PR	–	8	–	A	C
30	6.3	Chicken	2013	PR	CRO, CTX	8	CTX-M-8	A	C
20	9.6	Chicken	2013	PR	CRO, CTX, CTF, CPM, ENO	8	CTX-M-8	A	C
10	2.6	Chicken	2013	PR	CRO, CTX, TET	8	CTX-M-15	A	D
0	3.6	Chicken	2013	PR	TET	8	–	B1	E
	5.5	Chicken	2013	PR	CRO, CTX, CTF, 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, CTF, CPM, CIP, ENO	8	CTX-M-8	B1	H
	11.8	Chicken	2013	PR	CRO, CTX, CTF, CPM, 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 3.515 to 30.825.

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: ceftazidime; CFX: cefoxitin; CIP: ciprofloxacin; CLO: chloramphenicol; CPM: cefepime; CRO: ceftriaxone; CTF: ceftiofur; 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).

TABLE

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

Source ^a		Years of isolate collection	Enterobacteriaceae isolates tested <i>n</i>	Enterobacteriaceae isolates with growth on screening plates (2 mg/L colistin) <i>n</i> ^b	Isolates positive for <i>mcr-1</i> <i>N</i> (% 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 *mcr-1* polymerase chain reaction and sequencing [6].

strains belonged to the phylogroup A and five to the phylogroup B1. Clonal relatedness of the strains were further determined by *Xba*I 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).

The 16 *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) *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 *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.

References

1. Dropa M, Lincopan N, Balsalobre LC, Oliveira DE, Moura RA, Fernandes MR, et al. Genetic background of novel sequence types of CTX-M-8- and CTX-M-15-producing *Escherichia coli* and *Klebsiella pneumoniae* from public wastewater treatment plants in São Paulo, Brazil. *Environ Sci Pollut Res Int*.

- 2016;23(5):4953-8. DOI: 10.1007/s11356-016-6079-5 PMID: 26782324
2. Pereira GH, Garcia DO, Mostardeiro M, Ogassavara CT, Levin AS. Spread of carbapenem-resistant *Klebsiella pneumoniae* in a tertiary hospital in Sao Paulo, Brazil. *J Hosp Infect.* 2011;79(2):182-3. DOI: 10.1016/j.jhin.2011.05.023 PMID: 21798628
3. Silva KC, Moreno M, Cabrera C, Spira B, Cerdeira L, Lincopan N, et al. First Characterization of CTX-M-15-Producing *Escherichia coli* Strains Belonging to Sequence Type (ST) 410, ST224, and ST1284 from Commercial Swine in South America. *Antimicrob Agents Chemother.* 2016;60(4):2505-8. DOI: 10.1128/AAC.02788-15 PMID: 26824955
4. Casella T, Rodríguez MM, Takahashi JT, Ghiglione B, Dropa M, Assunção E, et al. Detection of blaCTX-M-type genes in complex class 1 integrons carried by Enterobacteriaceae isolated from retail chicken meat in Brazil. *Int J Food Microbiol.* 2015;197:88-91. DOI: 10.1016/j.ijfoodmicro.2014.12.001 PMID: 25576985
5. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters. Version 6.0, 2016. Växjö: EUCAST. Available from: http://www.eucast.org/clinical_breakpoints/
6. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis.* 2016;16(2):161-8. DOI: 10.1016/S1473-3099(15)00424-7 PMID: 26603172
7. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol.* 2000;66(10):4555-8. DOI: 10.1128/AEM.66.10.4555-4558.2000 PMID: 11010916
8. Skov RL, Monnet DL. Plasmid-mediated colistin resistance (mcr-1 gene): three months later, the story unfolds. *Euro Surveill.* 2016;21(9):30155. DOI: 10.2807/1560-7917.ES.2016.21.9.30155 PMID: 26967914
9. Elnahriry SS, Khalifa HO, Soliman AM, Ahmed AM, Hussein AM, Shimamoto T, et al. Emergence of plasmid-mediated colistin resistance gene mcr-1 in a clinical *Escherichia coli* isolate from Egypt. *Antimicrob Agents Chemother.* 2016;60(5):3249-50. DOI: 10.1128/AAC.00269-16 PMID: 26953204
10. Cannatelli A, Giani T, Antonelli A, Principe L, Luzzaro F, Rossolini GM. First detection of the mcr-1 colistin resistance gene in *Escherichia coli* in Italy. *Antimicrob Agents Chemother.* 2016;60(5):3257-8. DOI: 10.1128/AAC.00246-16 PMID: 26976865
11. Prim N, Rivera A, Rodríguez-Navarro J, Español M, Turbau M, Coll P, et al. Detection of mcr-1 colistin resistance gene in polyclonal *Escherichia coli* isolates in Barcelona, Spain, 2012 to 2015. *Euro Surveill.* 2016;21(13):30183. DOI: 10.2807/1560-7917.ES.2016.21.13.30183 PMID: 27055477
12. Arcilla MS, van Hattem JM, Matamoros S, Melles DC, Penders J, de Jong MD, et al. , COMBAT consortium. Dissemination of the mcr-1 colistin resistance gene. *Lancet Infect Dis.* 2016;16(2):147-9. DOI: 10.1016/S1473-3099(15)00541-1 PMID: 26711361
13. Du H, Chen L, Tang YW, Kreiswirth BN. Emergence of the mcr-1 colistin resistance gene in carbapenem-resistant Enterobacteriaceae. *Lancet Infect Dis.* 2016;16(3):287-8. DOI: 10.1016/S1473-3099(16)00056-6 PMID: 26842776
14. Kluytmans-van den Bergh MF, Huizinga P, Bonten MJ, Bos M, De Bruyne K, Friedrich AW, et al. Presence of mcr-1-positive Enterobacteriaceae in retail chicken meat but not in humans in the Netherlands since 2009. *Euro Surveill.* 2016;21(9):30149. DOI: 10.2807/1560-7917.ES.2016.21.9.30149 PMID: 26967540
15. Haenni M, Poirel L, Kieffer N, Châtre P, Saras E, Métayer V, et al. Co-occurrence of extended spectrum β lactamase and MCR-1 encoding genes on plasmids. *Lancet Infect Dis.* 2016;16(3):281-2. DOI: 10.1016/S1473-3099(16)00007-4 PMID: 26774244
16. Hasman H, Hammerum AM, Hansen F, Hendriksen RS, Olesen B, Agersø Y, et al. Detection of mcr-1 encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat, Denmark 2015. *Euro Surveill.* 2015;20(49):30085. DOI: 10.2807/1560-7917.ES.2015.20.49.30085 PMID: 26676364
17. Falgenhauer L, Waezsada SE, Yao Y, Mirzalioglu C, Käsbohrer A, Roesler U, et al. , RESET consortium. Colistin resistance gene mcr-1 in extended-spectrum β -lactamase-producing and carbapenemase-producing Gram-negative bacteria in Germany. *Lancet Infect Dis.* 2016;16(3):282-3. DOI: 10.1016/S1473-3099(16)00009-8 PMID: 26774242
18. Zurfluh K, Poirel L, Nordmann P, Nüesch-Inderbinen M, Hächler H, Stephan R. Occurrence of the Plasmid-Borne mcr-1 Colistin Resistance Gene in Extended-Spectrum- β -Lactamase-Producing Enterobacteriaceae in River Water and Imported Vegetable Samples in Switzerland. *Antimicrob Agents Chemother.* 2016;60(4):2594-5. DOI: 10.1128/AAC.00066-16 PMID: 26883696
19. Rossi F. The challenges of antimicrobial resistance in Brazil. *Clin Infect Dis.* 2011;52(9):1138-43. DOI: 10.1093/cid/cir120 PMID: 21467020
20. United States International Trade Commission (USITC). Brazil: Competitive Factors in Brazil Affecting U.S. and Brazilian Agricultural Sales in Selected Third Country Markets. Publication 4310. Washington, DC: USITC; 2012. Available online: <https://www.usitc.gov/publications/332/pub4310.pdf>
21. Morales AS, Fragoso de Araújo J, de Moura Gomes VT, Reis Costa AT, dos Prazeres Rodrigues D, Porfida Ferreira TS, et al. Colistin resistance in *Escherichia coli* and *Salmonella enterica* strains isolated from swine in Brazil. *ScientificWorldJournal.* 2012;2012:109795. DOI: 10.1100/2012/109795
22. Nordmann P, Poirel L. Plasmid-mediated colistin resistance: an additional antibiotic resistance menace. *Clin Microbiol Infect.* 2016;S1198-743X(16)30024-6. PMID: 27021419
23. Clinical and Laboratory Standard Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Informational Supplement. Wayne, PA: CLSI; 2007. CLSI document M100-S17.
24. European Committee on Antimicrobial Susceptibility Testing (EUCAST). EUCAST guidelines for detection of resistance mechanisms and specific resistance of clinical and/or epidemiological importance. Version 1.0, 2013. Växjö: EUCAST. Available from: <http://www.eucast.org/>
25. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Media preparation for EUCAST disk diffusion testing and for determination of MIC values by the broth microdilution method. Version 4.0, 2014. Växjö: EUCAST. Available from: <http://www.eucast.org/>

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