



## First report of *mcr-1*-harboring *Salmonella enterica* serovar Schwarzengrund isolated from poultry meat in Brazil

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### ABSTRACT

Brazilian poultry meat samples were screened for colistin-resistant *Salmonella enterica*. Sixty *Salmonella* isolates were tested for *in vitro* colistin resistance and *mcr-1*, *mcr-2*, *mcr-3* and *mcr-4* genes. Two isolates harbored the *mcr-1* gene and whole-genome analysis determined the serovar to be Schwarzengrund, ST96, harboring the IncX4 plasmid. This is the first report of *mcr-1*-harboring *Salmonella enterica* serovar Schwarzengrund in Brazil.

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The recent rise of colistin resistance and the rapid spread of *mcr-1*-harboring bacterial pathogens from humans and animals have become a major public health issue and require enhanced epidemiological surveillance. The intensive usage colistin in veterinary medicine promotes the maintenance and spread of foodborne *mcr-1*-positive isolates (Poirel and Nordmann, 2016).

Recently, poultry have been reported as an important reservoir of *mcr-1*-harboring *E. coli* worldwide (Lima Barbieri et al., 2017; Monte et al., 2017; Trung et al., 2017). However, poultry associated *mcr-1*-positive *Salmonella enterica* is less frequently reported. However, the *mcr-1* gene has been detected in low frequency from different *Salmonella* serotypes isolated from Portugal, Spain, France and England over the last 5 years (Doumith et al., 2016; Figueiredo et al., 2016; Quesada et al., 2016; Webb et al., 2016).

Here we present the evaluation of colistin resistance and the prevalence of *mcr-1*, *mcr-2*, *mcr-3* and *mcr-4* genes in *Salmonella enterica* strains isolated from poultry meat cuts sold at municipal markets, butchers and small markets of São Paulo city. A total of 60 strains, isolated between 2013 and 2016 from 33 poultry cuts of 24 markets,

were studied. Meat samples (wing = 27, thigh = 7, chest = 21, drumstick = 5) were submitted and processed in accordance to the methodologies described by Holt et al. (1994). *Salmonella enterica* identification was confirmed using biochemical tests and through *invA* gene amplification as previously described (Rahn et al., 1992). Serotyping was performed by the Enteric Pathogens Laboratory from Oswaldo Cruz Institute Foundation, Rio de Janeiro (FIOCRUZ-RJ).

Isolates were subjected to *mcr-1*, *mcr-2*, *mcr-3* and *mcr-4* PCR screening as previously described (Carattoli et al., 2017; Liu et al., 2016; Xavier et al., 2016; Yin et al., 2017). Colistin resistance was evaluated by minimal inhibitory concentrations (MIC) assessed through broth microdilution technique as recommended by the Clinical and Laboratory Standards Institute (M31-A3, 2008). The colistin-resistant strains were further evaluated against a panel of eight antimicrobial agents (ampicillin, ciprofloxacin, fosfomicin, gentamicin, tetracycline, ceftiofur, spectinomycin, florfenicol) by disk diffusion method.

From the 60 studied strains, seven demonstrated colistin MIC  $\geq 2$  mg/L and only two strains were positive for *mcr-1* gene (MIC = 8 mg/L). Among the colistin-resistant *Salmonella* isolates, none presented multidrug resistance phenotype; 71,4% were susceptible to all tested antimicrobials. The remaining isolates which presented colistin MIC ranging from 8 to 16 mg/L were resistant to at least one more antimicrobial class (Table 1).

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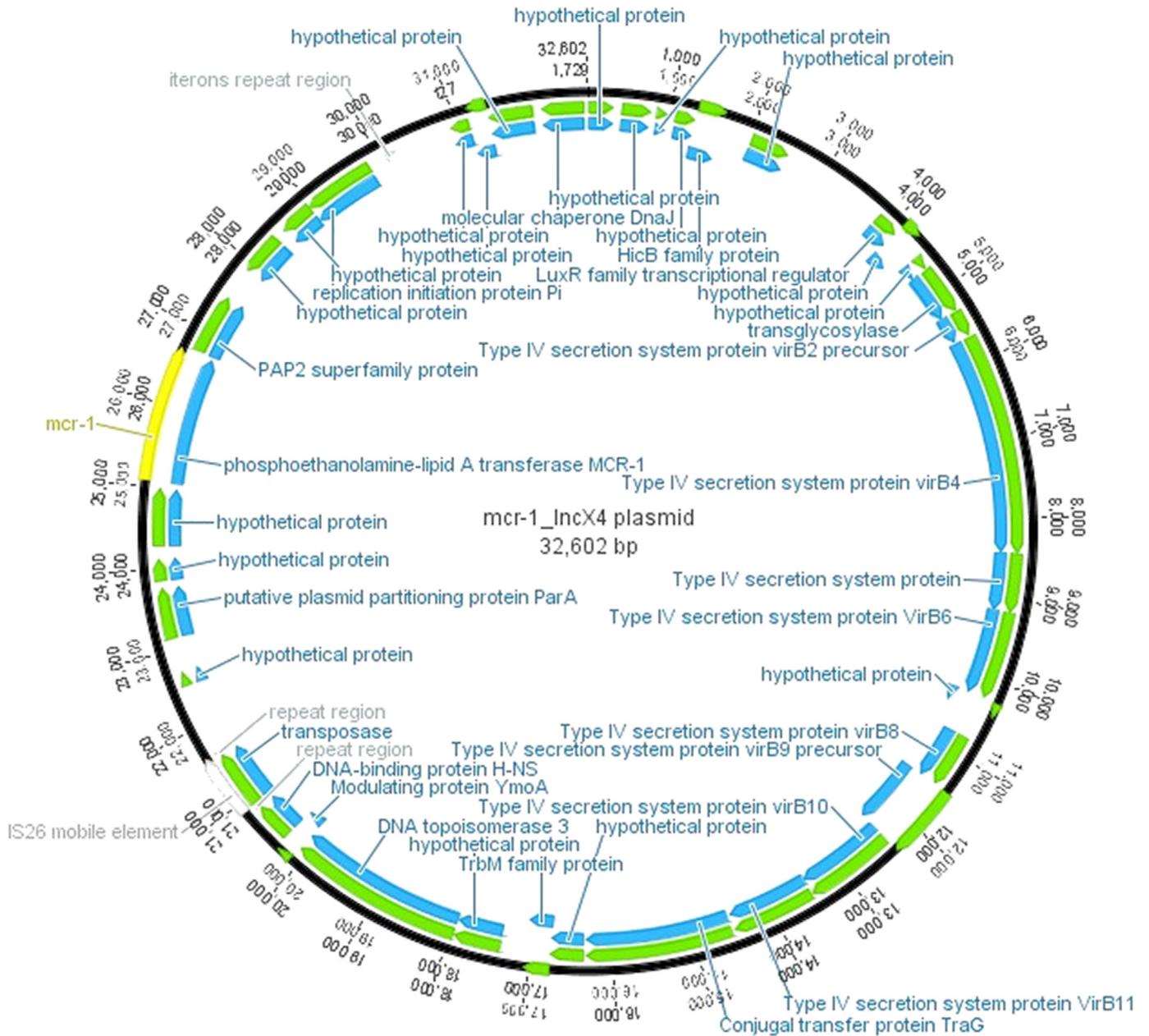
**Table 1**  
Colistin-resistant Brazilian *Salmonella enterica* strains isolated from poultry meat.

Strain	Cut	Market	Region	Serotype	Colistin MIC (mg/L)	Resistance profile*	PCR
A206–1.5	Chest	M52	South	Heidelberg	8	COL	-
A206–1.8	Chest	M52	South	Derby	8	COL	-
A265–1.1	Thigh	M67	West	Abony	8	COL	-
A268–1.1	Wing	M68	West	Abony	2	COL	-
A276–1.2	Wing	M70	West	Muenchen	8	COL / FLOR	-
A323–1.5	Chest	M82	North	Schwarzengrund	16	COL / FLOR	<i>mcr-1</i>
A323–1.11	Chest	M82	North	Schwarzengrund	16	COL	<i>mcr-1</i>

\* COL = colistin; FLOR = florfenicol.

Considering that the two *mcr-1*-positive isolates originated from the same meat sample, only the SA323 strain was selected for whole genome sequencing. Genomic DNA was purified with Illustra™ bacteria

genomicPrep Mini Spin Kit (GE Healthcare do Brasil Ltda, São Paulo, Brazil) and used for paired-end library preparation with Nextera™ DNA Sample Prep Kit (Illumina®) and sequencing through Illumina®



**Fig. 1.** Circular representation of the Brazilian *Salmonella enterica* serovar Schwarzengrund *mcr-1*-harboring IncX4 plasmid. In yellow, the *mcr-1* sequence. Green arrows indicate annotated genes and blue arrows the respective proteins. Numbers indicate nucleotide positions.

NextSeq platform. Base calling, trimming and *de novo* assembly were performed with CLC Main Workbench 7.5.1 (CLC Bio, QIAGEN). With genome coverage of ~300×, the assembly resulted in 23 scaffolds with a N<sub>50</sub> of 412,101 bp.

The SA323 draft genome (NIWS00000000) comprises ~4.6 Mb, with an overall G + C content of 52%. Automatic genome annotation was performed with NCBI Prokaryotic Genome Annotation Pipeline (Tatusova et al., 2016). The *in silico* multilocus sequence typing (MLST) analysis, serotyping and detection of acquired antibiotic resistance genes were performed using the Center for Genomic Epidemiology tools – MLST-1.8, SeqSero and ResFinder (Larsen et al., 2012; Zhang et al., 2015). The SA323 strain was characterized as ST96 and confirmed as serovar Schwarzengrund.

Scaffolds' ordering with reference strain *Salmonella enterica* serovar Schwarzengrund CVM19633 (GenBank accession No. CP001127) was performed with CLC Microbial Genomics Module (CLC Bio, QIAGEN) and demonstrated the existence of two extrachromosomal sequences that presented high identity with the *Escherichia coli* strain CSZ4 plasmid pCSZ4 (KX711706) and *Salmonella* serovar Anatum strain GT-01 plasmid PDM03 (CP013223).

The smaller extrachromosomal sequence (~3.3 kb) codifies only mobilization and hypothetical proteins similar to plasmids previously described in serovars Heidelberg and Anatum (Labbé et al., 2016; Marasini et al., 2016). The larger plasmid (32.6 kb) belongs to IncX4 group and presents a typical *mcr-1* cassette, with ParA and a hypothetical protein upstream from *mcr-1* gene that is followed by a PAP2 superfamily protein (Fig. 1). No other resistance genes were detected in the chromosome or the plasmids.

*Salmonella enterica* serovar Schwarzengrund has been reported as an emerging pathogen in Asia, Denmark and the United States since early 2000 (Aarestrup et al., 2007; Asai et al., 2009). The identification of multidrug-resistant clones disseminated among poultry and chicken meat with proven human transmission indicates the worldwide importance of this serovar (Aarestrup et al., 2007).

In Brazil, serovar Schwarzengrund has also been isolated from poultry and chicken meat (Tejada et al., 2016; Voss-Rech et al., 2015), but with few reports of antimicrobial resistance (Silva et al., 2013; Voss-Rech et al., 2015). Nevertheless, *Salmonella enterica* serovar Schwarzengrund had not yet been associated with colistin resistance or the respective *mcr* genes.

In Brazil, chicken meat had previously been identified as a reservoir for *mcr-1*-harboring *E. coli* isolates (Monte et al., 2017). However, recently, Rau et al. (2018) detected *mcr-1*-harboring *Salmonella enterica* serovar Typhimurium from retail frozen pork in southern Brazil. Our results highlight that commercial poultry meat is also an important reservoir of *mcr-1*-carrying *Enterobacteriaceae* and therefore it may be a risk for public health, contributing to the spread of *mcr* genes and also posing a greater threat to human health.

To date, all the plasmids harboring the *mcr-1* gene described in Brazil have been identified as belonging to the IncX4 family (Aires et al., 2017; Dalmolin et al., 2017; Fernandes et al., 2016; Monte et al., 2017; Sellera et al., 2017). The IncX4 plasmids have been previously detected in human and animal *Enterobacteriaceae*; besides being self-transferable, it was demonstrated that they can also be transferred between *Enterobacteriaceae* species (Sun et al., 2017). Regarding colistin resistance, the IncX4 plasmids have only been associated with *mcr-1* mobilization (Li et al., 2017) and appear to be key vectors for intercontinental dissemination of the *mcr* genes.

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## Conflict of Interests

The authors declare that they have no conflict of interests.

## References

- Aarestrup FM, Hendriksen RS, Lockett J, Gay K, Teates K, McDermott PF, et al. International spread of multidrug-resistant *Salmonella* Schwarzengrund in food products. *Emerg Infect Dis* 2007;13(5):726–31.
- Aires CAM, da Conceição-Neto OC, Tavares E, Oliveira TR, Dias CF, Montezzi LF, et al. Emergence of the plasmid-mediated *mcr-1* gene in clinical KPC-2-producing *Klebsiella pneumoniae* sequence type 392 in Brazil. *Antimicrob Agents Chemother* 2017;61. [pii: e00317–17].
- Asai T, Murakami K, Ozawa M, Koike R, Ishikawa H. Relationships between multidrug-resistant *Salmonella enterica* Serovar Schwarzengrund and both broiler chickens and retail chicken meats in Japan. *Jpn J Infect Dis* 2009;62:198–200.
- Caratoli A, Villa L, Feudi C, Curcio L, Orsini S, Luppi A, et al. Novel plasmid-mediated colistin resistance *mcr-4* gene in *Salmonella* and *Escherichia coli*, Italy 2013, Spain and Belgium, 2015 to 2016. *Euro Surveill* 2017;22. [pii: 30589].
- Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved Standard. CLSI document M31–A3, 3rd ed. Wayne (PA): Clinical and Laboratory Standards Institute; 2008.
- Dalmolin TV, Castro L, Mayer FQ, Zavascki AP, Martins AF, Lima-Morales D, et al. Co-occurrence of *mcr-1* and blaKPC-2 in a clinical isolate of *Escherichia coli* in Brazil. *J Antimicrob Chemother* 2017;72:2404–6.
- Doumith M, Godbole G, Ashton P, Larkin L, Dallman T, Day M, et al. Detection of the plasmid-mediated *mcr-1* gene conferring colistin resistance in human and food isolates of *Salmonella enterica* and *Escherichia coli* in England and Wales. *J Antimicrob Chemother* 2016;71:2300–5.
- Fernandes MR, McCulloch JA, Vianello MA, Moura Q, Pérez-Chaparro PJ, Esposito F, et al. First report of the globally disseminated IncX4 plasmid carrying the *mcr-1* gene in a colistin-resistant *Escherichia coli* sequence type 101 isolate from a human infection in Brazil. *Antimicrob Agents Chemother* 2016;60:6415–7.
- Figueiredo R, Card RM, Nunez J, Pomba C, Mendonça N, Anjum MF, et al. Detection of an *mcr-1*-encoding plasmid mediating colistin resistance in *Salmonella enterica* from retail meat in Portugal. *J Antimicrob Chemother* 2016;71:2338–40.
- Holt JG, Krieg NR, Sneath PHA. *Bergey's manual of determinative bacteriology*. Cap.5: Facultative anaerobic Gram-negative rods. 9 ed. Williams & Wilkins: Baltimore; 1994. p. 175–89.
- Labbé G, Edirmanasinghe R, Ziebell K, Nash JH, Bekal S, Parmley EJ, et al. Complete genome and plasmid sequences of three Canadian isolates of *Salmonella enterica* subsp. *enterica* Serovar Heidelberg from human and food sources. *Genome Announc* 2016;4. [pii: e01526–15].
- Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, et al. Multilocus sequence typing of total-genome-sequenced bacteria. *J Clin Microbiol* 2012;50:1355–61.
- Li R, Xie M, Zhang J, Yang Z, Liu L, Liu X, et al. Genetic characterization of *mcr-1*-bearing plasmids to depict molecular mechanisms underlying dissemination of the colistin resistance determinant. *J Antimicrob Chemother* 2017;72:393–401.
- Lima Barbieri N, Nielsen DW, Wannemuehler Y, Cavender T, Hussein A, S-g Yan, et al. *Mcr-1* identified in avian pathogenic *Escherichia coli* (APEC). *PLoS ONE* 2017;12. e0172997.
- 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;16:161–8.
- Marasini D, Abo-Shama UH, Fakhr MK. Complete genome sequences of *Salmonella enterica* Serovars Anatum and Anatum var. 15+, isolated from retail ground Turkey. *Genome Announc* 2016;4. [pii: e01619–15].
- Monte DF, Mem A, Fernandes MR, Cerdeira L, Esposito F, Galvão JA, et al. Chicken meat as a reservoir of Colistin-resistant *Escherichia coli* strains carrying *mcr-1* genes in South America. *Antimicrob Agents Chemother* 2017;61. [e02718–16].
- Poirer L, Nordmann P. Emerging plasmid-encoded colistin resistance: the animal world as the culprit? *J Antimicrob Chemother* 2016;71:2326–7.
- Quesada A, Ugarte-Ruiz M, Iglesias MR, Porrero MC, Martínez R, Florez-Cuadrado D, et al. Detection of plasmid mediated colistin resistance (MCR-1) in *Escherichia coli* and *Salmonella enterica* isolated from poultry and swine in Spain. *Res Vet Sci* 2016;105:134–5.
- Rahn K, De Grandis SA, Clarke RC, McEwen SA, Galán JE, Ginocchio C, et al. Amplification of an *invA* gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella*. *Mol Cell Probes* 1992;6:271–9.
- Rau RB, de Lima-Morales D, Wink PL, Ribeiro AR, Martins AF, Barth AL. Emergence of *mcr-1* producing *Salmonella enterica* serovar typhimurium from retail meat: first detection in Brazil. *Foodborne Pathog Dis* 2018;15:58–9.
- Sellera FP, Fernandes MR, Sartori L, Carvalho MP, Esposito F, Nascimento CL, et al. *Escherichia coli* carrying IncX4 plasmid-mediated *mcr-1* and blaCTX-M genes in infected migratory Magellanic penguins (*Spheniscus magellanicus*). *J Antimicrob Chemother* 2017;72:1255–6.
- Silva KC, Fontes LC, Moreno AM, Astolfi-Ferreira CS, Ferreira AJ, Lincopan N. Emergence of extended-spectrum-β-lactamase CTX-M-2-producing *Salmonella enterica* serovars

- Schwarzengrund and Agona in poultry farms. *Antimicrob Agents Chemother* 2013; 57:3458–9.
- Sun J, Fang L-X, Wu Z, Deng H, Yang RS, Li XP, et al. Genetic analysis of the IncX4 plasmids: implications for a unique pattern in the *mcr-1* acquisition. *Sci Rep* 2017;7:424.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, et al. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res* 2016;44: 6614–24.
- Tejada TS, Silva CSJ, Lopes NA, Silva DT, Agostinetto A, Silva EF, et al. DNA profiles of *Salmonella* Spp. isolated from chicken products and from broiler and human feces. *Rev Bras Cienc Avic* 2016;18:693–700.
- Trung NV, Matamoros S, Carrique-Mas JJ, Nghia NH, Nhung NT, Chieu TT, et al. Zoonotic transmission of *mcr-1* Colistin resistance gene from small-scale poultry farms, Vietnam. *Emerg Infect Dis* 2017;23:529–32.
- Voss-Rech D, Vaz CS, Alves L, Coldebella A, Leão JA, Rodrigues DP, et al. A temporal study of *Salmonella enterica* serotypes from broiler farms in Brazil. *Poult Sci* 2015;94:433–41.
- Webb HE, Granier SA, Marault M, Millemann Y, den Bakker HC, Nightingale KK, et al. Dissemination of the *mcr-1* colistin resistance gene. *Lancet* 2016;16:144–5.
- Xavier BB, Lammens C, Ruhel R, Kumar-Singh S, Butaye P, Goossens H, et al. Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016. *Euro Surveill* 2016;21. <https://doi.org/10.2807/1560-7917.ES.2016.21.27.30280>.
- Yin W, Li H, Shen Y, Liu Z, Wang S, Shen Z, et al. . Novel plasmid-mediated colistin resistance gene *mcr-3* in *Escherichia coli*. *MBio* 2017;8. [pii: e00543–17].
- Zhang S, Yin Y, Jones MB, Zhang Z, Deatherage Kaiser BL, Dinsmore BA, et al. *Salmonella* serotype determination utilizing high-throughput genome sequencing data. *J Clin Microbiol* 2015;53:1685–92.