Infection, Genetics and Evolution 96 (2021) 105156

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

# Colistin-resistant *mcr-1*-positive *Escherichia coli* ST1775-*H*137 co-harboring $bla_{CTX-M-2}$ and $bla_{CMY-2}$ recovered from an urban stream

João Pedro Rueda Furlan [a], Ralf Lopes [a], Micaela Santana Ramos [a], Lucas David Rodrigues dos Santos [a], Rafael da Silva Rosa [a], Eduardo Angelino Savazzi [b], Eliana Guedes Stehling [a,*]

[a] Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo (USP) - Ribeirão Preto, Brazil
[b] Companhia Ambiental do Estado de São Paulo (CETESB), Ribeirão Preto, Brazil

ARTICLE INFO

*Keywords:*
*Escherichia coli*
Colistin resistance
*mcr-1*
Extended-spectrum β-lactamase
Plasmid-mediated AmpC β-lactamase
Water
One Health

ABSTRACT

The rapid dissemination of colistin resistance *mcr*-type genes and extended-spectrum β-lactamase-encoding genes at the human-animal-environment interface has raised concerns worldwide. In this study, we performed a genomic investigation of a multidrug (MDR)- and colistin-resistant *Escherichia coli* strain recovered from an urban stream strongly affected by pollution and used for recreational purposes in Brazil. *E. coli* strain EW827 was resistant to clinically significant antimicrobials, including polymyxins, extended-spectrum cephalosporins, and fluoroquinolones. Whole-genome sequencing analysis revealed that EW827 strain belonged to ST1775 and carried the *fimH*137 allele, clinically relevant antimicrobial resistance genes (e.g., *mcr-1.1*, $bla_{CTX-M-2}$, and $bla_{CMY-2}$), tolerance genes to metals, and biocide resistance genes. Moreover, IncX4 and IncI1-ST12 replicon types were identified carrying *mcr-1.1* and $bla_{CMY-2}$, respectively. A novel genetic environment of the *mcr-1.1* gene, in which a 258-bp ΔIS5-like was inserted in the opposite orientation upstream of the *mcr-1.1-pap2* element, was also detected. Additionally, the $bla_{CTX-M-2}$ gene was harbored by a Tn*21*-like element on the chromosome. The occurrence of MDR *E. coli* co-harboring *mcr-1.1*, $bla_{CTX-M-2}$, and $bla_{CMY-2}$ in urban water represents a potential risk to humans, animals, and environmental safety. Therefore, epidemiological studies are required to monitoring multidrug-resistant bacteria and their antimicrobial resistance genes in aquatic ecosystems to determine possible routes and fates of these genes.

## 1. Introduction

The occurrence of multidrug-resistant (MDR) *E. coli* in the environment, including in aquatic ecosystems, has been increasingly reported, which is closely linked to the One Health approach for genomic surveillance studies on antimicrobial resistance at the human-animal-environment interface (McEwen and Collignon, 2018). In addition, the rapid dissemination of colistin resistance *mcr*-type genes has raised concerns worldwide since colistin is used to treat infections caused by MDR bacteria, mainly carbapenem-resistant Enterobacterales (Liu et al., 2016; Poirel et al., 2017). In this regard, *E. coli* strains are considered the main hosts of the *mcr-1* gene at the human-animal-environment interface. The *mcr-1* gene is commonly carried by plasmids, whereas its presence on the chromosome has been occasionally reported (Ling et al., 2020). IncX4, IncI2, and IncHI2 replicon types have accounted the majority of reported *mcr-1*-bearing plasmids (Nang et al., 2019).

Extended-spectrum β-lactamase (ESBL)- and plasmid-mediated AmpC β-lactamase (pAmpC)-encoding genes have been mainly identified in bacterial strains isolated from poultry and their derivatives causing resistance to extended-spectrum cephalosporins (ESCs) (Castellanos et al., 2019). Among these genes, $bla_{CTX-M-2}$ and $bla_{CMY-2}$ have been proposed to be originated from *Kluyvera* sp. and *Citrobacter freundii*, respectively, and disseminated throughout the Enterobacterales (Humeniuk et al., 2002; Jacoby, 2009). The $bla_{CTX-M-2}$ gene has been highlighted mainly in South America, while the $bla_{CMY-2}$ has been described worldwide (Bevan et al., 2017; Roer et al., 2019).

In this context, the increasing dissemination of clinically relevant antimicrobial resistance genes to the environment is a worrying public

* Corresponding author at: Av. do Café, s/n, Monte Alegre, Ribeirão Preto 14040-903, Brazil.
*E-mail address:* elianags@usp.br (E.G. Stehling).

https://doi.org/10.1016/j.meegid.2021.105156
Received 2 August 2021; Received in revised form 4 November 2021; Accepted 19 November 2021
Available online 23 November 2021

and environmental health problem. Therefore, we performed a genomic investigation of a *mcr-1*-positive *E. coli* strain exhibiting an MDR profile to clinically significant antimicrobials and co-harboring $bla_{CTX-M-2}$ and $bla_{CMY-2}$ genes isolated from an urban stream in Brazil, highlighting a real risk of human and animal exposure to antimicrobial-resistant bacteria and their resistance genes.

## 2. Material and methods

### 2.1. Water samples and bacterial isolation

During a surveillance study conducted in February and March 2020 to monitor the presence of colistin-resistant Gram-negative bacteria in rivers and streams located in the state of São Paulo, Brazil, water samples (1 L) were collected by the Environmental Company of São Paulo State. All samples were transported at 4 °C to the Laboratory of Environmental Microbiology, where this study was carried out. The water samples were filtered using sterile membrane filters with a pore size of 0.45 μm. Subsequently, the membrane filters were placed on MacConkey agar (Oxoid Ltd., United Kingdom) supplemented with colistin (2 mg/L) (Sigma-Aldrich, USA) and incubated for 24 h at 37 °C. Lactose-positive colonies were selected and stocked at −80 °C using Brain Heart Infusion broth (Oxoid Ltd., United Kingdom) supplemented with glycerol (15%). The strains were identified by sequencing of the 16S rRNA gene (Weisburg et al., 1991).

### 2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by disk diffusion, broth microdilution, and/or Etest methods following the CLSI (CLSI; M100, 30th, 2020) and BrCast (BrCast; v.10.0, 2020) guidelines. The antimicrobials tested were colistin, ampicillin, ampicillin/sulbactam, ceftriaxone, ceftazidime, cefepime, imipenem, meropenem, aztreonam, ciprofloxacin, gentamicin, sulfamethoxazole/trimethoprim, tetracycline, and chloramphenicol. Additionally, ESBL production was detected by the double-disk synergy test (Jarlier et al., 1988). The MDR profile was defined as resistant to at least one antimicrobial of three or more different categories (Magiorakos et al., 2012).

### 2.3. Detection of antimicrobial resistance genes

Antimicrobial resistance genes were screened by conventional PCR for *mcr*-type (*mcr-1* to *mcr-9*) and $bla_{CTX-M}$ groups [Group 1 (e.g., $bla_{CTX-M-1}$, $bla_{CTX-M-3}$, and $bla_{CTX-M-15}$); Group 2 (e.g., $bla_{CTX-M-2}$); Group 8/25 (e.g., $bla_{CTX-M-8}$ and $bla_{CTX-M-25}$); Group 9 (e.g., $bla_{CTX-M-9}$ and $bla_{CTX-M-14}$)] (Dallenne et al., 2010; Liu et al., 2016; Lescat et al., 2018; Borowiak et al., 2020).

### 2.4. Whole-genome sequencing (WGS), assembly, and annotation

Total DNA was extracted using the PureLink™ Genomic DNA Mini Kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. Whole-genome sequencing was carried out using the Illumina HiSeq 4000 (2 × 150 bp) platform (Illumina, USA). Reads with a Phred quality score < 20 were removed and adapters were trimmed using BBDuk, a tool of the BBMap package (https://github.com/BioInfoTools/BBMap). *De novo* genome assembly was performed by SPAdes v.3.9 (https://cge.cbs.dtu.dk/services/SPAdes/) and the annotation was carried out using Prokka v.1.14.5 (https://github.com/tseemann/prokka).

### 2.5. Genome analysis and phylogeny

The sequence type (ST), *fimH*-type, clonotype, and serotype were determined using MLST v.2.0, FimTyper v.1.0, CHTyper v.1.0, and SerotypeFinder v.2.0, respectively. Resistome and virulome were analyzed using ResFinder v.4.1 and VirulenceFinder v.2.0, respectively. Mobile genetic elements and their relation to resistome and virulome were identified by MobileElementFinder v.1.0.3. These tools are available at Center for Genomic Epidemiology (CGE) (http://www.genomicepidemiology.org/).

Phylogenetic group was determined using ClermonTyping (http://clermontyping.iame-research.center/). In addition, a minimum spanning tree based on cgMLST scheme was constructed using the MSTree v.2 algorithm in Enterobase (https://enterobase.warwick.ac.uk/species/index/ecoli) and visualized with iTOL v.5.7 (https://itol.embl.de/).

### 2.6. Plasmid assembly and analysis

Plasmid contigs were assembled by plasmidSPAdes v.3.15.0 (https://github.com/ablab/spades). BLASTn analysis was carried out and the gaps were closed according to Cerdeira et al. (2011). Annotation was performed by Rapid Annotations using Subsystems Technology server (https://rast.nmpdr.org/rast.cgi) and was manually curated using the Geneious v.2021.0.3 program (Biomatters Ltd., New Zealand). Analysis of transposable elements was performed by ISfinder (https://isfinder.biotoul.fr/). Multiple plasmid alignment was performed using the Easyfig program (https://mjsull.github.io/Easyfig/files.html). Plasmid replicon types and multilocus sequence typing were determined using PlasmidFinder v.2.1 and pMLST v.2.0, respectively, available at CGE.

### 2.7. Conjugation assays

Conjugation assays were carried out using azide-resistant *E. coli* C600 as recipient strain (Furlan et al., 2020). Transconjugants were selected using MacConkey agar (Oxoid Ltd., United Kingdom) supplemented with sodium azide (200 mg/L) and colistin (2 mg/L), or sodium azide (200 mg/L) and ceftriaxone (2 mg/L), and confirmed by conventional PCR for detection of *mcr*-type and β-lactamase-encoding genes, with further MIC evaluation.

## 3. Results

### 3.1. Mcr-1- and ESBL-producing E. coli

In this study, 45 strains were recovered from rivers and streams in Brazil. Among the isolates, 40 were classified as MDR, of which one *E. coli* strain, named EW827, showed resistance to colistin and ESCs and was selected for genomic investigation. The EW827 strain was recovered from Tanquinho Stream (21°08′34.4"S 47°48′09.5"W) in the city of Ribeirão Preto. The EW827 strain exhibited a MDR profile, including resistance to ampicillin (MIC ≥ 256 mg/L), ampicillin/sulbactam (≥ 32/16 mg/L), ceftazidime (16 mg/L), ceftriaxone (64 mg/L), cefepime (32 mg/L), aztreonam (32 mg/L), gentamicin (≥ 16 mg/L), ciprofloxacin (16 mg/L), tetracycline (≥ 64 mg/L), trimethoprim/sulfamethoxazole (≥ 4/76 mg/L), chloramphenicol (32 mg/L), and colistin (4 mg/L), but it was susceptible to imipenem (1 mg/L) and meropenem (1 mg/L). In addition, the EW827 strain was positive for the double-disk synergy test, suggesting production of ESBL. Subsequently, $bla_{CTX-M-2}$ and *mcr-1* genes were detected by conventional PCR.

### 3.2. Molecular typing and phylogenetic analysis

WGS revealed that *E. coli* strain EW827 (GenBank accession no. JAGIYM000000000) belonged to ST1775/CC350, phylogroup E, and serotype ONT:H4. This strain carried the *fimH*137 allele and was assigned to the clonotype CH31–137. Genomic relatedness analysis based on cgMLST revealed that EW827 was closely related to four other publicly available assembled genomes of *E. coli* E-ST1775-*H*137 isolated from humans and animals. Furthermore, *E. coli* E-ST1775-*H*137 from Kenya, Brazil and the United States differed from SCP19–26 from

Netherlands by 141 to 201-loci variant (Fig. 1).

### 3.3. Resistome

Resistome analysis detected resistance genes to colistin (*mcr-1.1*), β-lactams ($bla_{CTX-M-2}$, $bla_{CMY-2}$), tetracyclines (*tetA*), aminoglycosides (*aadA1*), folate pathway antagonist (*sul1*), phenicols (*catA1*), and macrolides [*mdf(A)*], as well as mutation in the quinolone resistance-determining region of GyrA (Ser83Leu). Comparative analysis showed that *mdf(A)*, *sul1*, *sul2*, *aph(6)-Id*, *aph(3")-Ib*, and *tetA* were the most prevalent among *E. coli* E-ST1775-*H*137 (Fig. 1). Tolerance genes to metals, including mercury (*merRTPCADE*), copper (*cueOR*, *copCD*, *cutACEF*), tellurium (*tehAB*, *terC*), nickel (*nikABCDE*), zinc (*zraP*), copper/silver (*cusSRCFBA*), nickel/cobalt/iron (*rcnABR*), cobalt/magnesium/manganese (*corA*), and tellurium/selenium/chromium (*ruvB*) were also identified. In addition, biocide resistance genes to hydrogen peroxide (*sitABCD*, *cpxA*, *fetAB*), formaldehyde (*frmA*), quaternary ammonium compounds (*sugE*, *mdtABCEFGHKNOP*, *emrABEKRY*), and organic solvents (*marRAB*) were also detected in the EW827 strain.

### 3.4. Virulence determinants

Virulome analysis showed several virulence determinants, including *iutA* (ferric aerobactin receptor), *iucC* (aerobactin synthetase), *iroN* (enterobactin siderophore receptor protein), *hlyF* (hemolysin F), *hra* (heat-resistant agglutinin), *cvaC* (microcin C), *estC* (putative type I secretion outer membrane protein), *mchF* (ABC transporter protein MchF), *gad* (glutamate decarboxylase), *iss* (increased serum survival), *ompT* [outer membrane protease (protein protease 7)], *traT* (outer membrane protein complement resistance), *chuA* (outer membrane hemin receptor), *sitA* (iron transport protein), and *tsh* (temperature-sensitive hemagglutinin). The *gad*, *iss*, and *chuA* genes were shared among all strains used in the phylogenetic analysis, whereas *hlyF*, *ompT*, and *traT* were found in three strains and *cvaC*, *mchF*, and *tsh* only in the EW827 strain (Fig. 1).

### 3.5. Plasmidome, genetic environments of mcr-1.1, $bla_{CMY-2}$ and $bla_{CTX-M-2}$ genes, and horizontal transfer

IncX4, IncI1-ST12, IncF [F18:A1:B1], ColRNAI, and Col(MG828) replicon types were identified in the EW827 strain. The IncX4 plasmid, named pEW827-MCR-1 (GenBank accession no. MW836072), and the IncI1-ST12 plasmid, named pEW827-CMY-2 (GenBank accession no. MW836073), carried *mcr-1.1* and $bla_{CMY-2}$, respectively.

The pEW827-MCR-1 plasmid was 33,613 bp in length, containing 41.9% GC and 44 coding regions (CDSs). Curiously, this plasmid harbored a novel genetic environment of the *mcr-1.1* gene, in which an ΔIS*5*-like (258 bp) was inserted in the opposite orientation upstream of the *mcr-1.1-pap2* element. Moreover, pEW827-MCR-1 showed a high nucleotide identity with other IncX4 plasmids of *E. coli* strains obtained from humans, animals (penguin and pork), and the environment (seawater and wastewater) in Brazil, Switzerland, Japan, China, and Taiwan (Fig. 2). On the other hand, a partial sequence of the pEW827-CMY-2 plasmid was obtained from assembly and was 66,883 bp in length, containing 48.4% GC and 71 CDSs. Analysis of the genetic environment of the $bla_{CMY-2}$ gene revealed the presence of the most prevalent context of this gene in IncI1-ST12 plasmids (IS*Ecp1*-$bla_{CMY-2}$-*blc-sugE*) (Fig. 3A).

In addition, the pEW827-MCR-1 and pEW827-CMY-2 plasmids harbored genes related to conjugative transfer, replication, maintenance, antimicrobial resistance, and insertion elements. Conjugation assays confirmed the transfer of both plasmids to azide-resistant *E. coli* C600, as confirmed by the *mcr-1*- or $bla_{CMY-2}$-positive transconjugants exhibiting resistance to colistin and cephalosporins, respectively.

In contrast, genome analysis revealed that the $bla_{CTX-M-2}$ gene was harbored by a Tn*21*-like element (GenBank accession no. MW836074) on the chromosome (Fig. 3B). The Tn*21*-like element was 29,179 bp in length and carried, in addition to $bla_{CTX-M-2}$, genes related to transposition, *eamA* (EamA transporter), *tetA*, *tetR*, *aadA1*, *sul1*, insertion sequences, EAL domain-containing protein (Urf2), and *merRTPCADE*, Additionally, the *catA1* gene was associated with an insertion sequence IS*1*-like upstream of the Tn*21*-like element. Insertion sequences IS*1*-like were located upstream and downstream of the Tn*21*-like element and may be responsible for its acquisition on the chromosome. Comparative analysis showed that the Tn*21*-like element in EW827 strain was most related to the Tn*21*-like element associated with In117 (59% query coverage and 99.98% nucleotide identity) and to the Tn*21*-like element on the plasmid RCS58_p (62% query coverage and 99.98% nucleotide identity) of *E. coli* strains isolated from humans in Spain and France, respectively (Fig. 3B).

## 4. Discussion

In this study, we report the presence of a colistin-resistant *mcr-1*-positive *E. coli* co-harboring $bla_{CTX-M-2}$ and $bla_{CMY-2}$ in a Brazilian urban stream. The Tanquinho Stream is strongly affected by pollution from industrial and commercial zones and is also used for recreational purposes, which is worrying (Grieco et al., 2017). In fact, in aquatic environments, domestic, industrial, and hospital effluent disposal contribute to the presence and spread of antimicrobial-resistant and metal-tolerant bacteria and their genes. In this regard, the acquisition and transmission of antimicrobial resistance genes can frequently occur in these environments (Marti et al., 2014). Additionally, international high-risk clones harboring *mcr-1* and ESBL-encoding genes have disseminated in aquatic environments (Fernandes et al., 2017; Zhu et al., 2020). The presence of bacteria resistant to colistin harboring the *mcr-1* gene in the environment is a risk to humans and animals, posing a threat to public health (Ovejero et al., 2017).

*E. coli* ST1775 strains have been reported in humans, animals and foods. However, there are no reports of their occurrence in the environment so far. Colistin-resistant *mcr-1*-positive *E. coli* ST1775 strains from a human and retail chicken meat were reported in Germany and Switzerland, respectively (Donà et al., 2017; Wise et al., 2018). Moreover, ESCs-resistant CMY-2-producing *E. coli* ST1775 strains were

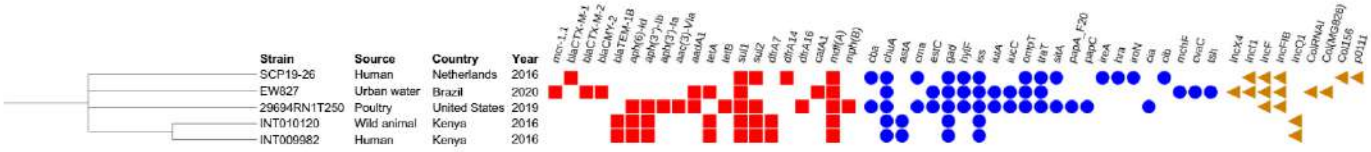


**Fig. 1.** Minimum spanning tree based on cgMLST of *E. coli* E-ST1775-*H*137 strains showing the source of isolation, country, collection year, antimicrobial resistance genes, virulence determinants, and replicon types. Red squares, blue circles, and mustard yellow triangles indicate antimicrobial resistance genes, virulence determinants, and replicon types, respectively. Assembled genomes of *E. coli* strains SCP19–26 (BioSample accession no. SAMEA4429509), EW827 (this study; BioSample accession no. SAMN18543060), 29694RN1T250 (Barcode no. ESC_RA0902AA), INT010120 (BioSample accession no. SAMEA5611283), and INT009982 (BioSample accession no. SAMEA5611263) are available at EnteroBase (http://enterobase.warwick.ac.uk/species/index/ecoli). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

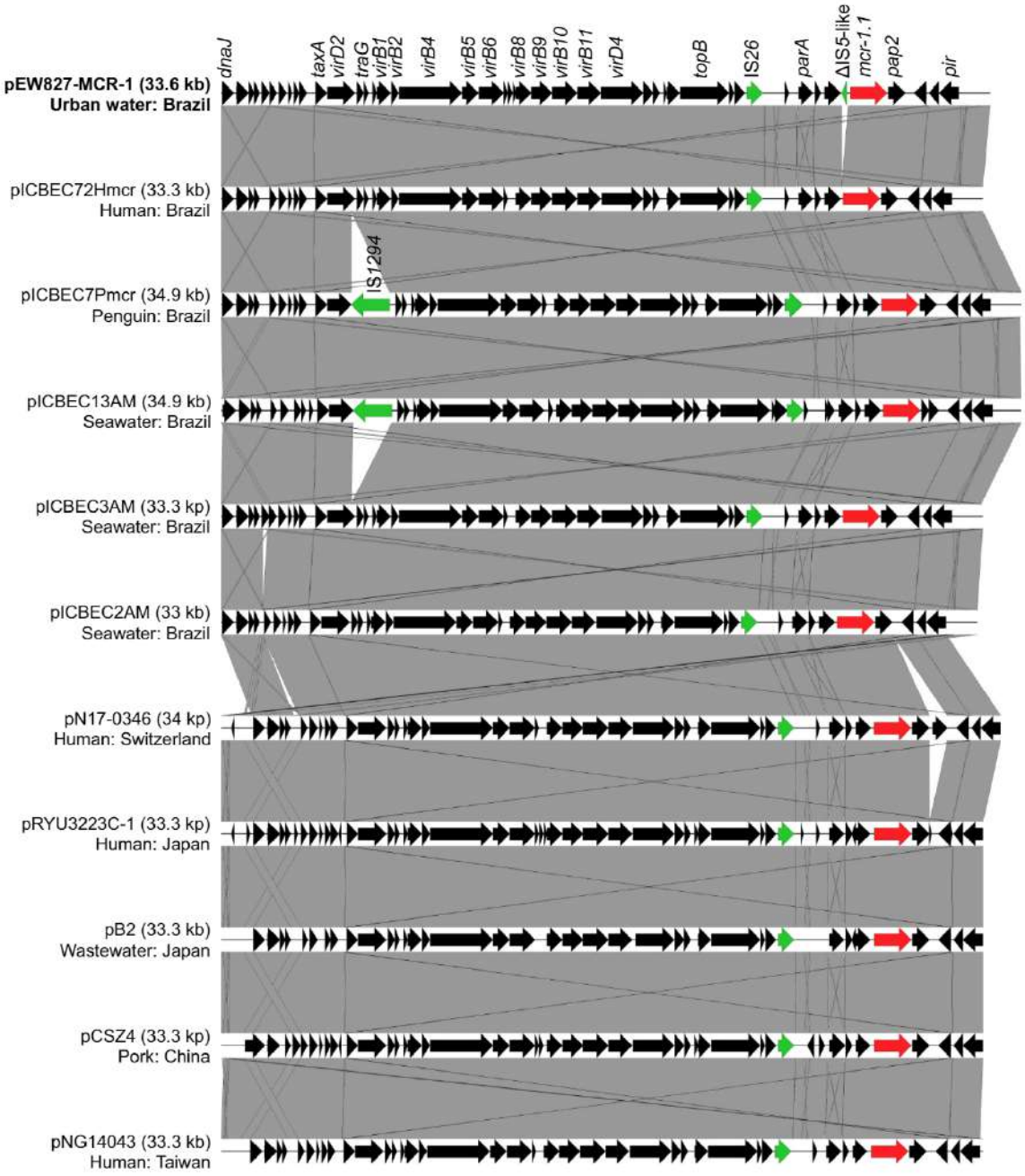


**Fig. 2.** Full-length alignment of IncX4 plasmids carrying the *mcr-1.1* gene in *E. coli* isolates from urban water (pEW827-MCR-1, GenBank accession no. MW836072), humans (pICEBC72Hmcr, GenBank accession no. CP015977; pN17–0346, GenBank accession no. CP031291; pRYU3223C-1, GenBank accession no. AP018411; pNG14043, GenBank accession no. KY120364), penguin (pICBEC7Pmcr, GenBank accession no. CP017246), pork (pCSZ4, GenBank accession no. KX711706), seawater (pICBEC13AM, GenBank accession no. KY770025; pICBEC3AM, GenBank accession no. KY770024; pICBEC2AM, GenBank accession no. KY770023), and wastewater (pB2, GenBank accession no. LC479085). Red, green, and black arrows indicate the *mcr-1.1* gene, insertion elements, and other genes, respectively. The gray shading represents shared regions of homology. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

described in a poultry chain in Colombia and broiler flock in Denmark (Agersø et al., 2014; Castellanos et al., 2017), whereas the $bla_{CTX\text{-}M\text{-}2}$ gene was reported in ST1775 strains obtained from chicken cloacal swabs and chicken meats in Brazil (Casella et al., 2018). Curiously, none of the *E. coli* ST1775 strains were previously reported carrying *mcr-1* and $bla_{CTX\text{-}M\text{-}2}$, as was observed in the EW827 strain.

Regarding resistance plasmids, IncX4 plasmids have a conserved ~30 kb genetic backbone and it is one of the main plasmids involved in the global dissemination of the *mcr-1* gene, which is commonly bordered by *pap2* encoding transmembrane protein (Sun et al., 2017). In South America, poultry meat is considered an emerging reservoir of *mcr-1*/IncX4 in colistin-resistant *E. coli* (Monte et al., 2017). Besides, in Brazil, *mcr-1*/IncX4 have been identified at the human-animal-environment interface, highlighting its presence in seawater of public beaches (Fernandes et al., 2016; Sellera et al., 2017); however, in the macroregion of Ribeirão Preto, where this study was carried out, there are no reports of the *mcr-1* gene in aquatic ecosystems. The presence of *mcr-1*/IncX4 in a colistin-resistant *E. coli* strain isolated from an urban stream reinforces the important role of IncX4 plasmids in the dissemination of the *mcr-1* gene.

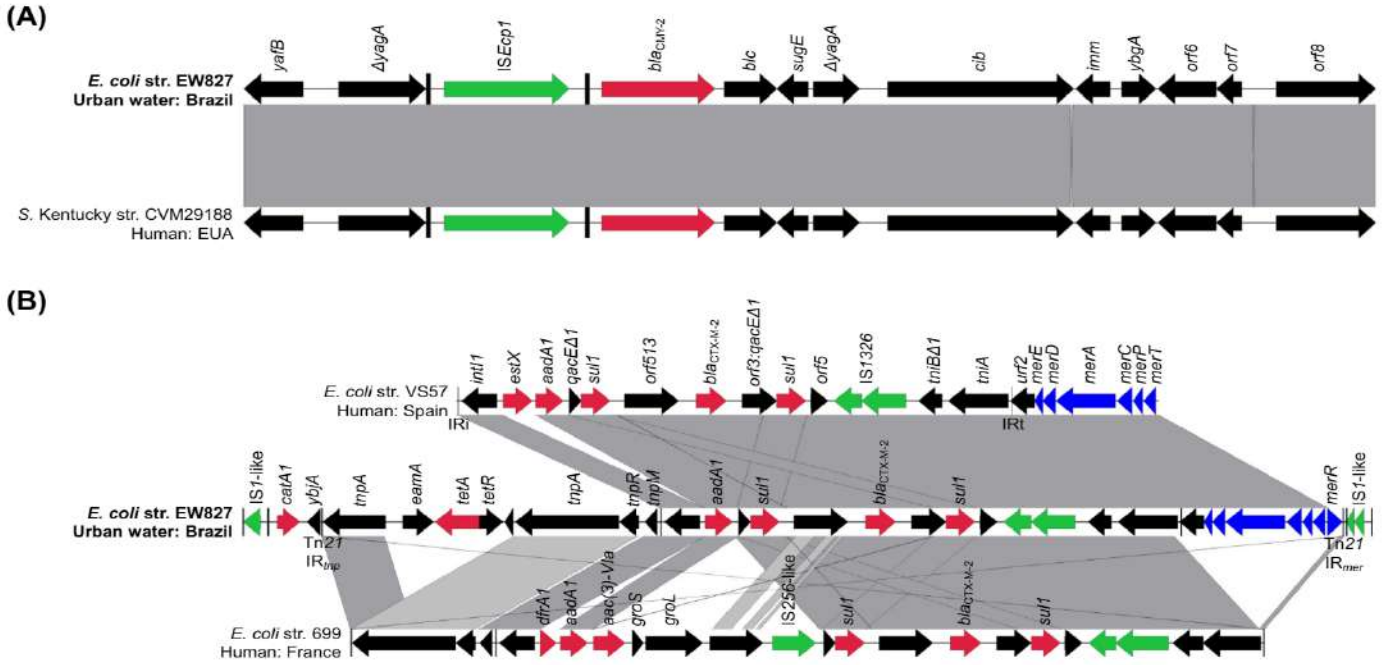


**Fig. 3.** Genetic environments of $bla_{CMY-2}$ and $bla_{CTX-M-2}$ genes. (A) Comparison between genetic environments of $bla_{CMY-2}$ in IncI1-ST12 plasmids. Plasmids pEW827-CMY-2 (GenBank accession no. MW836073) and pCVM29188_101 (GenBank accession no. NC_011077) of *E. coli* and *Salmonella* Kentucky, respectively. (B) Comparison between genetic environments of $bla_{CTX-M-2}$. The $bla_{CTX-M-2}$ gene associated with Tn*21*-like elements in *E. coli* strains isolated from urban water (this study; GenBank accession no. MW836074) and humans in Spain (GenBank accession no. DQ125241) and France (GenBank accession no. LT985268). Vertical bars represent inverted repeats (IRs); $IR_{tnp}$ and $IR_{mer}$ delimit the Tn*21*-like element. Red, green, blue, and black arrows indicate antimicrobial resistance genes, insertion elements, mercury tolerance genes, and other genes, respectively. The gray shading represents shared regions of homology. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The $bla_{CMY-2}$ gene is the most described pAmpC, which has been associated with IncI1/ST12 (~ 100 kb) found in *E. coli* strains from humans and poultry (Castellanos et al., 2019; Roer et al., 2019). The $bla_{CMY-2}$ gene has been mobilized onto plasmids of different replicon types, highlighting IncC and IncI1 (Bortolaia et al., 2014; Chen et al., 2019). In general, IS*Ecp1* element has been identified upstream of the $bla_{CMY-2}$ gene and also composes the most prevalent genetic environment (IS*Ecp1*-$bla_{CMY-2}$-*blc*-*sugE*-*encR*) in plasmids (Pietsch et al., 2018). In Brazil, a MDR *E. coli* strain co-harboring $bla_{CTX-M-2}$ and $bla_{CMY-2}$/IncI1-ST12 was reported from poultry (Cunha et al., 2017).

In contrast, the co-occurrence of *mcr-1* and $bla_{CTX-M-like}$ genes in *E. coli* strains have been reported worldwide, including in South America (Zhang et al., 2016; Fernandes et al., 2017). In this regard, *mcr-1* and $bla_{CTX-M-like}$ genes can also be co-located on the same plasmid, such as IncHI2 plasmid, which can accelerate their dissemination (Grami et al., 2016; Zhang et al., 2019). The $bla_{CTX-M-2}$ gene has usually been harbored in plasmids, whereas chromosomal integration of this gene has been less frequently reported (Minarini et al., 2009; Ferreira et al., 2014). In addition, IS*CR1* elements (also called Orf513) have been described in complex class 1 integrons and transposons and are involved in the mobilization of the $bla_{CTX-M-2}$ gene (Arduino et al., 2003; Gillings et al., 2009).

## 5. Conclusion

The occurrence of MDR- and colistin-resistant *E. coli* co-harboring *mcr-1.1*, ESBL- and pAmpC-encoding genes in urban water represents a potential risk to humans, animals and environmental safety and calls for attention to the dissemination of antimicrobial-resistant pathogens and their antimicrobial resistance genes in the environment. In fact, urban residents and animals could be exposed to contaminated water and, consequently, to infections and/or bacterial colonization. To the best of our knowledge, this is the first report of an *E. coli* ST1775 strain recovered from an environmental source and co-harboring *mcr-1.1*, $bla_{CTX-M-2}$ and $bla_{CMY-2}$. Therefore, epidemiological studies are required to monitoring multidrug-resistant bacteria in aquatic ecosystems, focusing on clinically relevant antimicrobial resistance genes, in order to determine possible routes for these genes.

## Ethical approval

Not required.

## Declaration of Competing Interest

The authors declare no conflicts of interest.

## Acknowledgements

This study was supported by São Paulo Research Foundation - FAPESP [grant number 2018/19539-0]. The authors thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) [grant no. 2018/01890-3], the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) [grant no. 88882.180855/2018-01, 88887.464733/2019-00, 88887.314388/2019-00, 88887.519091/2020-00, and Finance code 001], and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) [grant no. 130086/2021-5] for fellowships.

## References

Agersø, Y., Jensen, J.D., Hasman, H., Pedersen, K., 2014. Spread of extended spectrum cephalosporinase-producing *Escherichia coli* clones and plasmids from parent animals to broilers and to broiler meat in a production without use of cephalosporins. Foodborne Pathog. Dis. 11, 740–746. https://doi.org/10.1089/fpd.2014.1742.

Arduino, S.M., Catalano, M., Orman, B.E., Roy, P.H., Centrón, D., 2003. Molecular epidemiology of orf513-bearing class 1 integrons in multiresistant clinical isolates

from Argentinean hospitals. Antimicrob. Agents Chemother. 47, 3945–3949. https://doi.org/10.1128/aac.47.12.3945-3949.2003.

Bevan, E.R., Jones, A.M., Hawkey, P.M., 2017. Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. J. Antimicrob. Chemother. 72, 2145–2155. https://doi.org/10.1093/jac/dkx146.

Borowiak, M., Baumann, B., Fischer, J., Thomas, K., Deneke, C., Hammerl, J.A., Szabo, I., Malorny, B., 2020. Development of a novel *mcr-6* to *mcr-9* multiplex PCR and assessment of *mcr-1* to *mcr-9* occurrence in colistin-resistant *Salmonella enterica* isolates from environment, feed, animals and food (2011-2018) in Germany. Front. Microbiol. 11, 80. https://doi.org/10.3389/fmicb.2020.00080.

Bortolaia, V., Hansen, K.H., Nielsen, C.A., Fritsche, T.R., Guardabassi, L., 2014. High diversity of plasmids harbouring $bla_{CMY-2}$ among clinical *Escherichia coli* isolates from humans and companion animals in the upper Midwestern USA. J. Antimicrob. Chemother. 69, 1492–1496. https://doi.org/10.1093/jac/dku011.

Casella, T., Haenni, M., Madela, N.K., Andrade, L.K., Pradela, L.K., Andrade, L.N., Darini, A., Madec, J.Y., Nogueira, M., 2018. Extended-spectrum cephalosporin-resistant *Escherichia coli* isolated from chickens and chicken meat in Brazil is associated with rare and complex resistance plasmids and pandemic ST lineages. J. Antimicrob. Chemother. 73, 3293–3297. https://doi.org/10.1093/jac/dky335.

Castellanos, L.R., Donado-Godoy, P., León, M., Clavijo, V., Arevalo, A., Bernal, J.F., Timmerman, A.J., Mevius, D.J., Wagenaar, J.A., Hordijk, J., 2017. High heterogeneity of *Escherichia coli* sequence types harbouring ESBL/AmpC genes on IncI1 plasmids in the Colombian poultry chain. PLoS One 12, e0170777. https://doi.org/10.1371/journal.pone.0170777.

Castellanos, L.R., van der Graaf-van Bloois, L., Donado-Godoy, P., Mevius, D.J., Wagenaar, J.A., Hordijk, J., Zomer, A.L., 2019. Phylogenomic investigation of IncI1-Iγ plasmids harboring $bla_{CMY-2}$ and $bla_{SHV-12}$ in *Salmonella enterica* and *Escherichia coli* in multiple countries. Antimicrob. Agents Chemother. 63 https://doi.org/10.1128/AAC.02546-18 e02546–18.

Cerdeira, L.T., Carneiro, A.R., Ramos, R.T., de Almeida, S.S., D'Afonseca, V., Schneider, M.P., Baumbach, J., Tauch, A., McCulloch, J.A., Azevedo, V.A., Silva, A., 2011. Rapid hybrid de novo assembly of a microbial genome using only short reads: *Corynebacterium pseudotuberculosis* I19 as a case study. J. Microbiol. Methods 86, 218–223. https://doi.org/10.1016/j.mimet.2011.05.008.

Chen, C.Y., Hsieh, P.H., Chang, C.Y., Yang, S.T., Chen, Y.H., Chang, K., Lu, P.L., 2019. Molecular epidemiology of the emerging ceftriaxone resistant non-typhoidal *Salmonella* in southern Taiwan. J. Microbiol. Immunol. Infect. 52, 289–296. https://doi.org/10.1016/j.jmii.2018.08.007.

Cunha, M.P., Lincopan, N., Cerdeira, L., Esposito, F., Dropa, M., Franco, L.S., Moreno, A. M., Knöbl, T., 2017. Coexistence of CTX-M-2, CTX-M-55, CMY-2, FosA3, and QnrB19 in Extraintestinal pathogenic *Escherichia coli* from poultry in Brazil. Antimicrob. Agents Chemother. 61 https://doi.org/10.1128/AAC.02474-16 e02474–16.

Dallenne, C., Da Costa, A., Decré, D., Favier, C., Arlet, G., 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in *Enterobacteriaceae*. J. Antimicrob. Chemother. 65, 490–495. https://doi.org/10.1093/jac/dkp498.

Donà, V., Bernasconi, O.J., Pires, J., Collaud, A., Overesch, G., Ramette, A., Perreten, V., Endimiani, A., 2017. Heterogeneous genetic location of *mcr-1* in colistin-resistant *Escherichia coli* isolates from humans and retail chicken meat in Switzerland: emergence of *mcr-1*-carrying IncK2 plasmids. Antimicrob. Agents Chemother. 61 https://doi.org/10.1128/AAC.01245-17 e01245–17.

Fernandes, M.R., McCulloch, J.A., Vianello, M.A., Moura, Q., Pérez-Chaparro, P.J., Esposito, F., Sartori, L., Dropa, M., Matté, M.H., Lira, D.P., Mamizuka, E.M., Lincopan, N., 2016. 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. 60, 6415–6417. https://doi.org/10.1128/AAC.01325-16.

Fernandes, M.R., Sellera, F.P., Esposito, F., Sabino, C.P., Cerdeira, L., Lincopan, N., 2017. Colistin-resistant *mcr-1*-positive *Escherichia coli* on public beaches, an infectious threat emerging in recreational waters. Antimicrob. Agents Chemother. 61 https://doi.org/10.1128/AAC.00234-17 e00234–17.

Ferreira, J.C., Penha Filho, R.A.C., Andrade, L.N., Berchieri, A., Darini, A.L.C., 2014. Detection of chromosomal *bla*(CTX-M-2) in diverse *Escherichia coli* isolates from healthy broiler chickens. Clin. Microbiol. Infect. 20, O623–O626. https://doi.org/10.1111/1469-0691.12531.

Furlan, J.P.R., Lopes, R., Gonzalez, I.H.L., Ramos, P.L., von Zeska Kress, M.R., Stehling, E.G., 2020. Hypermucoviscous/hypervirulent and extensively drug-resistant QnrB2-, QnrS1-, and CTX-M-3-coproducing *Klebsiella pneumoniae* ST2121 isolated from an infected elephant (*Loxodonta africana*). Vet. Microbiol. 251, 108909 https://doi.org/10.1016/j.vetmic.2020.108909.

Gillings, M.R., Labbate, M., Sajjad, A., Giguère, N.J., Holley, M.P., Stokes, H.W., 2009. Mobilization of a Tn*402*-like class 1 integron with a novel cassette array via flanking miniature inverted-repeat transposable element-like structures Appl. Environ. Microbiol. 75, 6002–6004. https://doi.org/10.1128/AEM.01033-09.

Grami, R., Mansour, W., Mehri, W., Bouallègue, O., Boujaâfar, N., Madec, J.Y., Haenni, M., 2016. Impact of food animal trade on the spread of *mcr-1*-mediated colistin resistance, Tunisia, July 2015. Euro Surveill. 21, 30144. https://doi.org/10.2807/1560-7917.ES.2016.21.8.30144.

Grieco, A.A., Fregonesi, B.M., Tonani, K.A.A., Silva, T.V., Celere, B.S., Trevilato, T.M.B., Segura-Muñoz, S.I., Alves, R.I.S., 2017. Spatial and temporal diagnosis of the physicochemical and microbiological conditions of Tanquinho's stream, Ribeirão Preto, SP, Brazil. Rev. Ambient. Água. 12, 282–298. https://doi.org/10.4136/ambi-agua.1837.

Humeniuk, C., Arlet, G., Gautier, V., Grimont, P., Labia, R., Philippon, A., 2002. Beta-lactamases of *Kluyvera ascorbata*, probable progenitors of some plasmid-encoded CTX-M types. Antimicrob. Agents Chemother. 46, 3045–3049. https://doi.org/10.1128/aac.46.9.3045-3049.2002.

Jacoby, G.A., 2009. AmpC beta-lactamases. Clin. Microbiol. Rev. 22, 161–182. https://doi.org/10.1128/CMR.00036-08.

Jarlier, V., Nicolas, M.H., Fournier, G., Philippon, A., 1988. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. Rev. Infect. Dis. 10, 867–878. https://doi.org/10.1093/clinids/10.4.867.

Lescat, M., Poirel, L., Nordmann, P., 2018. Rapid multiplex polymerase chain reaction for detection of *mcr-1* to *mcr-5* genes. Diagn Microbiol. Infect. Dis. 92, 267–269. https://doi.org/10.1016/j.diagmicrobio.2018.04.010.

Ling, Z., Yin, W., Shen, Z., Wang, Y., Shen, J., Walsh, T.R., 2020. Epidemiology of mobile colistin resistance genes *mcr-1* to *mcr-9*. J. Antimicrob. Chemother. 75, 3087–3095. https://doi.org/10.1093/jac/dkaa205.

Liu, Y.Y., Wang, Y., Walsh, T.R., Yi, L.X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., Huang, X., Yu, L.F., Gu, D., Ren, H., Chen, X., Lv, L., He, D., Zhou, H., Liang, Z., Liu, J.H., Shen, J., 2016. 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. 16, 161–168. https://doi.org/10.1016/S1473-3099(15)00424-7.

Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D.L., Rice, L.B., Stelling, J., Struelens, M.J., Vatopoulos, A., Weber, J.T., Monnet, D.L., 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. 18, 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x.

Marti, E., Variatza, E., Balcazar, J.L., 2014. The role of aquatic ecosystems as reservoirs of antibiotic resistance. Trends Microbiol. 22, 36–41. https://doi.org/10.1016/j.tim.2013.11.001.

McEwen, S.A., Collignon, P.J., 2018. Antimicrobial resistance: a one health perspective. Microbiol. Spectr. 6, 2. https://doi.org/10.1128/microbiolspec.ARBA-0009-2017.

Minarini, L.A., Poirel, L., Trevisani, N.A., Darini, A.L., Nordmann, P., 2009. Predominance of CTX-M-type extended-spectrum beta-lactamase genes among enterobacterial isolates from outpatients in Brazil. Diagn. Microbiol. Infect. Dis. 65, 202–206. https://doi.org/10.1016/j.diagmicrobio.2009.05.021.

Monte, D.F., Mem, A., Fernandes, M.R., Cerdeira, L., Esposito, F., Galvão, J.A., Franco, B., Lincopan, N., Landgraf, M., 2017. Chicken meat as a reservoir of colistin-resistant *Escherichia coli* strains carrying *mcr-1* genes in South America. Antimicrob. Agents Chemother. 61 https://doi.org/10.1128/AAC.02718-16 e02718–16.

Nang, S.C., Li, J., Velkov, T., 2019. The rise and spread of *mcr* plasmid-mediated polymyxin resistance. Crit. Rev. Microbiol. 45, 131–161. https://doi.org/10.1080/1040841X.2018.1492902.

Ovejero, C.M., Delgado-Blas, J.F., Calero-Caceres, W., Muniesa, M., Gonzalez-Zorn, B., 2017. Spread of *mcr-1*-carrying *Enterobacteriaceae* in sewage water from Spain. J. Antimicrob. Chemother. 72, 1050–1053. https://doi.org/10.1093/jac/dkw533.

Pietsch, M., Irrgang, A., Roschanski, N., Brenner Michael, G., Hamprecht, A., Rieber, H., Käsbohrer, A., Schwarz, S., Rösler, U., Kreienbrock, L., Pfeifer, Y., Fuchs, S., Werner, G., RESET Study Group, 2018. Whole genome analyses of CMY-2-producing *Escherichia coli* isolates from humans, animals and food in Germany. BMC Genomics 19, 601. https://doi.org/10.1186/s12864-018-4976-3.

Poirel, L., Jayol, A., Nordmann, P., 2017. Polymyxins: antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. Clin. Microbiol. Rev. 30, 557–596. https://doi.org/10.1128/CMR.00064-16.

Roer, L., Overballe-Petersen, S., Hansen, F., Johannesen, T.B., Stegger, M., Bortolaia, V., Leekitcharoenphon, P., Korsgaard, H.B., Seyfarth, A.M., Mossong, J., Wattiau, P., Boland, C., Hansen, D.S., Hasman, H., Hammerum, A.M., Hendriksen, R.S., 2019. ST131 *fimH22 Escherichia coli* isolate with a $bla_{CMY-2}$/IncI1/ST12 plasmid obtained from a patient with bloodstream infection: highly similar to *E. coli* isolates of broiler origin. J. Antimicrob. Chemother. 74, 557–560. https://doi.org/10.1093/jac/dky484.

Sellera, F.P., Fernandes, M.R., Sartori, L., Carvalho, M.P., Esposito, F., Nascimento, C.L., Dutra, G.H., Mamizuka, E.M., Pérez-Chaparro, P.J., McCulloch, J.A., Lincopan, N., 2017. *Escherichia coli* carrying IncX4 plasmid-mediated *mcr-1* and $bla_{CTX-M}$ genes in infected migratory Magellanic penguins (*Spheniscus magellanicus*). J. Antimicrob. Chemother. 72, 1255–1256. https://doi.org/10.1093/jac/dkw543.

Sun, J., Fang, L.-X., Wu, Z., Deng, H., Yang, R.-S., Li, X.-P., Li, S.-M., Liao, X.-P., Feng, Y., Liu, Y.-H., 2017. Genetic analysis of the IncX4 plasmids: implications for a unique pattern in the *mcr-1* acquisition. Sci. Rep. 7, 1–9. https://doi.org/10.1038/s41598-017-00095-x.

Weisburg, W.G., Barns, S.M., Pelletier, D.A., Lane, D.J., 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. 173, 697–703. https://doi.org/10.1128/jb.173.2.697-703.1991.

Wise, M.G., Estabrook, M.A., Sahm, D.F., Stone, G.G., Kazmierczak, K.M., 2018. Prevalence of *mcr*-type genes among colistin-resistant *Enterobacteriaceae* collected in 2014-2016 as part of the INFORM global surveillance program. PLoS One 13, e0195281. https://doi.org/10.1371/journal.pone.0195281.

Zhang, H., Seward, C.H., Wu, Z., Ye, H., Feng, H., 2016. Genomic insights into the ESBL and MCR-1-producing ST648 *Escherichia coli* with multi-drug resistance. Sci. Bull. 61, 875–878. https://doi.org/10.1007/s11434-016-1086-y.

Zhang, P., Wang, J., Wang, X., Bai, X., Ma, J., Dang, R., Xiong, Y., Fanning, S., Bai, L., Yang, Z., 2019. Characterization of five *Escherichia coli* isolates co-expressing ESBL and MCR-1 resistance mechanisms from different origins in China. Front. Microbiol. 10, 1994. https://doi.org/10.3389/fmicb.2019.01994.

Zhu, L., Zhou, Z., Liu, Y., Lin, Z., Shuai, X., Xu, L., Chen, H., 2020. Comprehensive understanding of the plasmid-mediated colistin resistance gene *mcr-1* in aquatic environments. Environ. Sci. Technol. 54, 1603–1613. https://doi.org/10.1021/acs.est.9b05919.