Archives of Current Research International

Volume 24, Issue 12, Page 389-403, 2024; Article no.ACRI.128736
ISSN: 2454-7077

Detection of *mcr-1* and *mcr-2* Genes among *Escherichia coli* and *Klebsiella pneumoniae* with One Health Approach for Antimicrobial Resistance in Brazil

Erika Alexandra Daza-Cardona [a,b*], Paulo de Tarso Teles Dourado de Aragão [c], Jhon Buenhombre [d], Guilherme Mendes Prado [a], Maria Nelly Cajiao-Pachón [d], Raquel Oliveira dos Santos Fontenelle [a] and Francisco Cesar Barroso Barbosa [a]

[a] Postgraduate Program in Health Sciences, Federal University of Ceará, No 62042-280, Sobral, Ceará, Brazil.
[b] Facultad de Medicina Veterinaria, Fundación Universitaria Agraria de Colombia, Cl 170 #54ª-10, Bogotá, Colombia.
[c] Faculty of Nursing, Maurício de Nassau College – Uninassau Sobral, No. 62042-230, Sobral, Ceará, Brazil.
[d] Especialización en Bienestar Animal y Etología, Fundación Universitaria Agraria de Colombia, Cl 170 #54ª-10, Bogotá, Colombia.

Authors' contributions

This work was carried out in collaboration among all authors. Authors EAD-C, RODSF and FCBB conceptualized the research work. Authors EAD-C, PDTTDDA, JB, GMP and RODSF did methodized the work. Authors EAD-C, PDTTDDA, JB and GMP did formal analysed and investigated. Authors EAD-C and JB wrote the original draft preparation. Authors EAD-C, PDTTDDA, JB, GMP, MNC-P, RODSF and FCBB wrote, reviewed and edited the manuscript. Authors RODSF and FCBB did supervised the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/acri/2024/v24i121030

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/128736

**Corresponding author: Email: erika.daza.cardona@gmail.com;*

Cite as: *Daza-Cardona, Erika Alexandra, Paulo de Tarso Teles Dourado de Aragão, Jhon Buenhombre, Guilherme Mendes Prado, Maria Nelly Cajiao-Pachón, Raquel Oliveira dos Santos Fontenelle, and Francisco Cesar Barroso Barbosa. 2024. "Detection of Mcr-1 and Mcr-2 Genes Among Escherichia Coli and Klebsiella Pneumoniae With One Health Approach for Antimicrobial Resistance in Brazil". Archives of Current Research International 24 (12):389-403. https://doi.org/10.9734/acri/2024/v24i121030.*

Original Research Article

Received: 28/10/2024
Accepted: 30/12/2024
Published: 30/12/2024

ABSTRACT

Nowadays, antimicrobial resistance is recognized as one of the most important global human health problems in the 21st century. Antimicrobial-resistant microorganisms can disseminate between ecosystems and have been found in humans, food, animals, plants and the environment. This study aimed to detect and characterize the *mcr* gene´s presence responsible for colistin resistance in Enterobacteriaceae isolates from humans, animals, the environment and food in Northeastern Brazil. The molecular identification of the *mcr-1* and *mcr-2* was carried out through the polymerase chain reaction (PCR), followed by an electrophoretic run. In isolates in which *mcr* genes were detected, the sensitivity profile for colistin was evaluated using the Broth Microdilution method. In total, 50 specimens of *Escherichia coli* from humans (n=14), poultry (n=19), cheese (n=12) and water (n=5) and 16 specimens of *Klebsiella pneumoniae* isolated from humans were analyzed. The *mcr-2* gene was not detected in any isolate. Three of the 30 *K. pneumoniae* isolates (10%) positives for the *mcr-1* gene were recovered from human clinical samples, one of the 19 *E. coli* isolates (5.3%) was retrieved from poultry, and one of the 12 *E. coli* isolates (8.3%) was recovered from cheese. The *E. coli* strains positive for the *mcr-1* gene showed a resistance profile to colistin sulfate with MIC of 4 µg/mL, and *K. pneumoniae* strains showed an intermediate profile to colistin (MIC < 0,25 µg/mL). Hence, these data unveil that enterobacteria from various sources can carry the *mcr-1* gene, conferring resistance to colistin, and that the gene is circulating in Northeastern Brazil. The discovered results make a crucial contribution to molecular and epidemiological surveillance within a One Health framework, aiming to prevent the dissemination of these genes both within and beyond the region.

Keywords: mcr gene; polymyxins; colistin; antimicrobial resistance; one health.

1. INTRODUCTION

Antimicrobial resistance (AMR) is recognized as one of the most critical global problems in the 21st century (Prestinaci, Pezzotti, & Pantosti 2015; Shad, 2018; B.-T. Liu et al., 2019; Owolabi & Azeez, 2020). It has been declared a significant threat to global health, with the potential to reverse advances in disease, treatment and impeding other global priorities, including human development (Yang & Buttery 2018). The AMR is an ecological problem characterized by complex interactions involving diverse microbial populations that affect the health of humans, animals and the environment. Therefore, it makes sense to address this problem both by analyzing the residues of these drugs in the environment, as well as by studying AMR, taking into account its complexity and ecological nature, using a coordinated and multi-sectorial approach, such as that of One Health (Harbarth et al., 2015; McEwen & Collignon 2018).

The One Health concept is particularly relevant and includes food safety, control of zoonoses and combating antibiotic resistance; it is based on the mutual interdependence of people and animals and the recognition that they share the same environment and many diseases and infections (Collignon & McEwen 2019). The before mentioned highlight the importance of an integrated and holistic One Health approach in combating AMR.

The use of antibiotics in livestock and aquaculture is common for growth enhancement, treatment and disease prevention and is likely to be a significant contributor to the global problem of antimicrobial resistance (Prestinaci, Pezzotti & Pantosti, 2015; Touati et al., 2019). For example, colistin is one of the antimicrobials choices for treating infections caused by microorganism's resistant to carbapenems. However, some countries have actively used this drug in animal production as a growth promoter (Y.-Y. Liu et al., 2016). As a result, colistin lost effectiveness due to spread of the *mcr* gene, which is a member of the phosphatidylethanolamine (pEtN) transferase family. The *mcr* gene encodes cytoplasmatic transmembrane proteins that transfers a pEtN residue to the lipid-A present in the cell membranes of Gram-negative bacteria (Feng et al., 2022; Anyanwu, Jaja & Nwobi 2020). To

date, ten slightly different variants of the *mcr-1* gene *(mcr*-1 to *mcr-10*) have been identified in various bacteria isolated from animals, foods, farms, humans, and the environment (Hussein et al., 2021). In Latin America and the Caribbean, the *mcr-1* gene is distributed across several countries, including Argentina, Brazil, Bolivia, Colombia, Chile, Ecuador, Paraguay, Peru, Uruguay, Venezuela (Conceição-Neto, O. C., Aires et al., 2017; Dominguez et al., 2017; European Medicines Agency, 2016) and México (Garza-Ramos et al., 2018). Additionally, the *mcr-4*, *mcr-3*, *mcr-7* and *mcr-9.1* genes have been identified in Brazil, and the *mcr-5* gene is present in Brazil, Colombia and Paraguay (Daza-Cardona et al., 2022; Kieffer et al., 2018; Nesporova et al., 2019; Wise et al., 2018; Costa-Júnior et al., 2023). Brazil, Bolivia, and Argentina have the highest number of *mcr*-positive isolates, while only Colombia (*mcr-5*) and Brazil (*mcr-3*) (Saavedra et al., 2016), *mcr-5* (Cyoia et al., 2019) exhibit *mcr* genes other than type 1. *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella enterica* serovar Typhimurium are the main carriers of the gene within the continent (Ugarte Silva et al., 2018; Ishii et al., 2018; Saavedra et al., 2016; Papa-Ezdra et al., 2019).

In Brazil, a high prevalence of the *mcr-1* gene has been identified in poultry isolates of *E. coli* (Barbieri et al., 2017). Additionally, the presence of the *mcr-1* gene has been documented in beef, swine, food items (specifically chicken meat), water sources, and ultimately, in humans (Palmeira et al., 2018). This indicates that animal husbandry practices may serve as a potential source of resistance within the human food chain, particularly in countries like Brazil where colistin is routinely used for animal health. This is especially significant in major animal protein-exporting nations (Saidenberg et al., 2020; Palmeira et al., 2018).

Since April 2016, in South America, the mechanism of resistance of the *mcr* gene has been identified in *E. coli* and other Enterobacteriaceae isolated from food, animal´s samples and clinical samples of symptomatic or asymptomatic patients (Medina, 2017; Barlaam et al., 2019). This gene was also detected in isolates obtained from agricultural soil (Lopes et al. 2021), marine environments (Cordeiro-Moura et al., 2022), mangroves (Sacramento et al., 2018), wild animals (Fuentes-Castillo et al., 2021) and surface water samples, showing environmental contamination (McEwen & Collignon, 2018).

Despite the global attention to AMR, studies regarding the mcr gene remain relatively scarce in developing countries with failures in health systems management, surveillance and control of antimicrobial resistance. Therefore, a One Health approach to genomic surveillance studies is required to effectively detect and limit the spread of antimicrobial-resistant bacteria and their resistance genes (Lopes et al. 2021). Therefore, this study aimed to investigate the occurrence of the *mcr-1* and *mcr-2* genes in bacteria isolated from clinical specimens in humans, poultry, food, and water in Northeastern Brazil.

2. MATERIALS AND METHODS

2.1 Bacterial Isolates

A total of 66 isolates were analyzed, human origin (n=30), poultry (n=19), food (cheese) (n=12) and environmental samples (water) (n=5). The clinical isolates from humans were collected in 2015, originating from a bronchoalveolar lavage and two tissue fragments from hospitalized individuals. The samples from the organs of broiler chickens were collected in 2020. Water samples were collected from water sources in the city of Sobral, while the food samples, consisting of artisanal cheeses, both collected between 2017 and 2018. Fifty out of the 66 isolates were specimens of *E. coli* from poultry (n=19), humans (n=14), cheese (n=12) and water for human consumption (n=5). The remaining 16 isolates were identified as *K. pneumoniae* isolated all obtained from human samples collected in Sobral City-CE, Brazil. The bacterial strains from human samples were sourced from the biological collections of the Microbiology Laboratory at the Federal University of Ceará (UFC)/Sobral Campus. The strains from other samples types were obtained from the Microbiology Laboratory at the State University of Vale do Acaraú (UVA).

2.2 Analysis of Susceptibility Profile

All isolates from human infections had their antimicrobial susceptibility profiles were analyzed using the automated VITEK 2® system (BioMérieux, Marcy-l'Étoile, France).

2.3 Plasmid DNA Extraction

For molecular analysis, all isolates were performed to alkaline lysis Miniprep method to extract plasmid DNA. Reagents: Solution I (10 mM EDTA pH 8.0), solution II (0.1 M NaOH, 1%

SDS) and solution III (250 g/L Potassium Acetate, 15% vol/vol Acetic Acid) were used for the extraction. Subsequently, quantification and quality assessment by 1.0% agarose gel electrophoresis stained in ethidium bromide was made.

2.4 Polymerase Chain Reaction (PCR) Analysis

All the isolates were screened for the presence of the *mcr-1* using the protocol reported by Liu et al. (Y.-Y. Liu et al. 2016) and for the presence of the *mcr-2* the protocol reported by Xavier et al., (2016). The genes were amplified using specific primers (Integrated DNA technologies®). For the *mcr-1* gene the primer sequences used were CLR5-F (5'-CGGTCAGTCCGTTTGTTC-3') and CLR5-R (5'-CTTGGTCGGTCTGTAGGG-3'). Moreover, the following primers designed to target the *mcr-2* were MCR2-IF (5'-TGTTGCTTGTGCCGATTGGA3') and MCR2-IR (5' AGATGGTATTGTTGGTTGCTG-3'). The cycling conditions used were as follows: initial denaturation (1 cycle) at 94°C 5 min, denaturation (35 cycles) at 94°C 30 sec, annealing at 55°C 30 sec, initial elongation at 72°C 30 sec and final extension cycle at 72° 5 min (Cavaco, Mordhorst & Hendriksen, 2016). Same parameters were used for the two reactions. PCR generated amplicons were run on a 1% agarose gel and stained in ethidium bromide to visualize the 309 bp product for the *mcr-1* and 567 bp for the *mcr-2* in the U.V. transilluminator (Enduro™ GDS Touch). The standard strains used in the PCR were CCBH20180 (*mcr-1*-positive) and CCBH35182 (*mcr-2*-positive), provided by the Oswaldo Cruz Foundation (Fiocruz).

2.5 Minimum Inhibitory Concentration (MIC) Test to Colistin by the Broth Microdilution Method (BMD)

For the isolates with the *mcr* gene, MIC was performed using the BMD with colistin sulfate (Sigma-Aldrich St. Louis, MO, USA) according to the Clinical Laboratory Standard Institute (CLSI) to assess the *in vitro* sensitivity to that antimicrobial.

The lowest concentration of antibiotic that was able to inhibit bacterial growth in the well plate microdilution was defined by spectrometry with an ELISA reader (Bio Trak II – Plate Reader) with an absorbance of 620 nm. Cut-off point to interpret these results for Colistin and Polymyxin B were used (Intermediate ≤ 2 µg/mL, Resistant ≥ 4 µg/mL) (Clinical and Laboratory Standards Institute 2020a).

3. RESULTS

The *mcr-1* was detected in five out of the 66 isolates (7.6%) evaluated in this study, as indicated by the respective amplification band (Fig. 1). The origin and identification of the isolates are detailed in Table 1. Three of the 30 isolates (10%) positives for the *mcr-1* gene were recovered from human clinical samples, one of the 19 isolates (5.3%) was retrieved from poultry, and one of the 12 isolates (8.3%) was recovered from cheese. Screening for the *mcr-2* gene did not detected its presence in any of the isolates tested.

The BMD test revealed that the positive samples for the *mcr-1* gene, 3/5 (60%) had an intermediate profile with MIC lower than 0.25 µg/mL and corresponded to isolates from nosocomial infections, and 2/5 (40%) had a colistin resistance profile with MIC of 4 µg/mL that corresponded to the poultry and cheese isolates (Table 2).

Antimicrobial susceptibility testing by BMD (VITEK 2® system) showed that the *mcr-1* positive nosocomial isolates exhibited susceptibility to colistin (MIC) < 0,5 mg/L. However, they exhibited a variable resistance profile to other antimicrobial agents and were producer of extended-spectrum β-lactamase (ESBL) (Table 3). For the *E. coli* isolates, no susceptibility testing was performed because the VITEK 2® system was located in a human clinical laboratory, and samples from different origins could not be processed.

Table 1. Origin for positive samples for the presence of the *mcr-1* gene

Origin	Isolates with *mcr-1*	Microorganism
Human	3	*K. pneumoniae*
Animal	1	*E. coli*
Food	1	*E. coli*

Table 2. MIC results by BMD method for colistin sulfate of *mcr-1* positive isolates

Isolate	MIC µg/mL	Profile (according CLSI, 2020)
E. coli ATCC 25922	0,25	
K. pneumoniae ATCC 700603	0,25	
K. pneumoniae Human ID H9	< 0,25	Intermediate
K. pneumoniae Human ID H17	< 0,25	Intermediate
K. pneumoniae Human ID H21	< 0,25	Intermediate
E. coli Animal ID AV 38	4	Resistant
E. coli Food ID AL 57	4	Resistant

Table 3. Susceptibility phenotypic profile of *mcr-1* positive nosocomial isolates

Patient	Date	Infection Site	Microorganism	Colistin MIC VITEK 2®	Antimicrobial susceptibility test
Human ID H9 (12.38816-4)	May 2015	Bronquial Lavage	*K. pneumoniae*	Susceptible < 0,5 mg/L	AMK: S, AMP: R, AMS: R, FEP: R, FOX: R, CAZ: R, CRO: R, CXM: R, CIP: R, **COL: S**, ETP: S, GEN: R, IPM: S, MEM: S, TZP: I, TGC: S, ESBL: POSITIVE
Human ID H17 (5900.0022.3912)	June 2015	Tissue Fragment	*K. pneumoniae*	Susceptible < 0,5 mg/L	AMK: S, AMP: R, AMS: R, FEP: R, FOX: I, CAZ: R, CRO: R, CXM: R, CIP: R, **COL: S**, ETP: S, GEN: S, IPM: S, MEM: S, TZP: R, TGC: S, ESBL: POSITIVE
Human ID H21 (5900.0022.3912)	June 2015	Tissue Fragment	*K. pneumoniae*	Susceptible < 0,5 mg/L	AMK: S, AMP: R, AMS: R, FEP: R, FOX: R, CAZ: R, CRO: R, CXM: R, CIP: R, **COL: S**, ETP: S, GEN: R, IPM: S, MEM: S, TZP: R, TGC: S, ESBL: POSITIVE

AMK, amikacin; AMP, ampicilina; AMS, ampicilin/sulbactam; FEP, cefepime; FOX, cefoxitin; CAZ, ceftazidime, CRO, ceftriaxone; CXM, cefuroxime; CIP, ciprofloxacin; COL, colistin; ETP, ertapenem; GEN, gentamicina; IPM, imipenem; MEM, meropenem; TZP, piperacilin/Tazobactam; TGC, tigeciclin; S, susceptible; I, intermediate; R, resistant

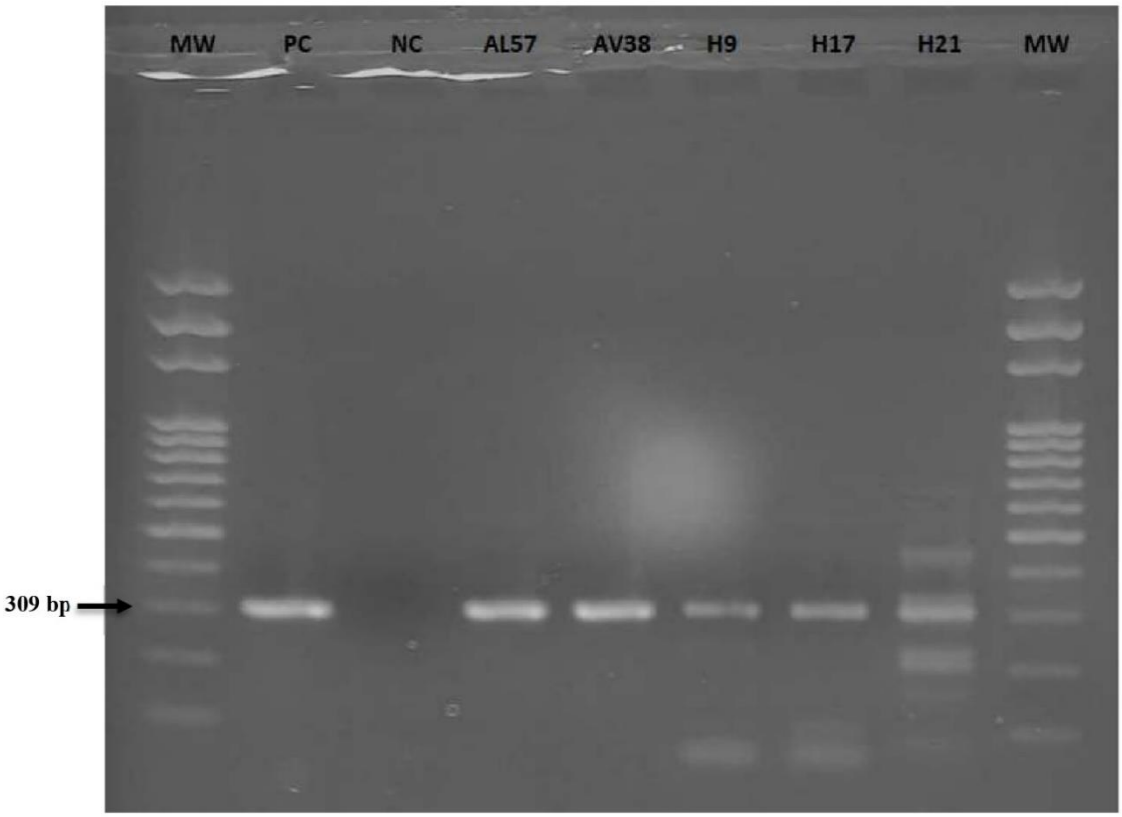

Fig. 1. Amplification products obtained by PCR performed for the detection of the *mcr-1*
MW: Molecular weight, PC: Positive control/standard strain (309 bp mcr-1 gene), NC: Negative control/sterile ultrapure water, AL57: Food isolate, AV38: Broiler isolate, H9: Nosocomial infection isolate No. 9, H17: Nosocomial infection isolate No. 17, H21: Nosocomial infection isolate No. 21

4. DISCUSSION

We have detected the presence of the *mcr-1* gene in clinical samples from humans, animals, and food (cheese) in Northeastern Brazil. Our findings align with previous studies conducted in Brazil and the region (V. Rocha, Paiva, and Lima 2019), confirming the presence of the *mcr-1* gene in various sources at the human-animal-environment interface (Daza-Cardona et al., 2022; Lopes et al., 2021; Monte et al., 2017; Yauri-Condor et al., 2020; V. Rocha, Paiva & Lima, 2019). This poses a major risk for the region, as resistant bacteria originating from humans, animals, and the environment can potentially spread from one country to another (Ramon-Pardo, Sati & Galas 2018).

Given that the human isolates date back to 2015, it is possible to correlate the gene´s dissemination in the environment and animals, as the samples were collected from locations in close proximity. The clinical environment is known to be a source of antibiotic resistance due to the extensive and intensive use of antibiotics, which creates natural selective pressure on bacteria (Jiménez Pearson et al., 2019; Prestinaci et al., 2015; C. Rocha, Reynolds & Simons, 2015).

Brazil has the highest number of reported data on *mcr*-positive bacteria in Latin America (V. Rocha, Paiva, and Lima 2019) and is a major exporter of animal protein (Saidenberg et al., 2020; Palmeira et al., 2018), which could facilitate the dissemination of antibiotic-resistant bacteria (Gelbíčová et al. 2019) throughout the region and potentially globally.

Regarding human samples, our results confirmed the presence of *K. pneumoniae* harboring the *mcr-1* gene in Northeastern Brazil, a gene that has been detected on all continents, as well as in isolates from humans, animals and the environment (M. Liu et al., 2024). Similar reports have been found in southern Brazil (Dalmolin et al., 2018; Aires et al., 2017) where high prevalence rates are reported for *K. pneumoniae*

have been observed in secretions (Mota, Oliveira & Souto, 2018). This highlights how *K. pneumoniae* has been implicated in both hospital-acquired and community-acquired human infections (M. Liu et al., 2024).

However, we did not find the presence of the gene in *E. coli*, despite the probability of co-colonization of *mcr-1*-harboring distinct species (Perdigão Neto et al., 2019) and the fact that most reports in Brazil have already documented the gene in *E. coli* isolates from wound infections, bloodstream infections, pneumonia in humans (Miriam R. Fernandes et al., 2016; I. V. Rocha et al., 2017; Aires et al., 2017).

Additionally, a similar gene, *mcr-1.1*, has been identified in the northeast region of Brazil in both *K. pneumoniae* and *E. coli* (I. Vasconcelos et al. 2020) as well as in the southern region in *E. coli* carrying an plasmid mediated *mcr-1* from community, healthcare-acquired infections and colonization (Paiva et al., 2021). Furthermore, *mcr-1.5* gene has been isolated from a human urinary tract infection (Fuga et al., 2024). Therefore, further screenings and surveillance are necessary to confirm the absence of *E. coli* harboring the *mcr-1* gene in humans in the northeastern region.

Concerning poultry, our *E. coli* isolates corroborate the presence and perhaps the circulation of the *mcr-1* gene in Brazilian poultry, as this has been reported since 2016 (M. R. Fernandes et al., 2016) in the south, southeast (Monte et al., 2017; M. R. Fernandes et al., 2016; Gelbíčová et al., 2019; Miriam R. Fernandes et al., 2016; Barbieri et al., 2017; Cyoia et al., 2019), west, north (Monte et al., 2017), and now in the northeast region, as reported by Vasconcelos et al., (2020) (I. Vasconcelos et al., 2020) for chicken carcasses and in our study for poultry. While our study specifically tested resistance to polymyxin, others, such as Cyoia et al., (2019) (Cyoia et al., 2019) and Saidenberg et al., (2020), reported that *E. coli* isolated from poultry harboring the *mcr-1* gene were also resistant to other antibiotics, indicating a profile of multidrug resistance (MDR) (Saidenberg et al. 2020). While Saidenberg et al., (2020) found almost twice the prevalence of our study, i.e., 9.37%; Cyoia et al., (2019) found a very low prevalence (Cyoia et al., 2019). The differences in prevalence's could be attributed to the varying sample sizes and colistin use between regions. Similarly, the presence of the gene has been found in other livestock such as cattle (Sacramento et al., 2018) and pigs (M. R. Fernandes et al., 2016) as well as in agricultural soil with a history of cow manure use (Lopes et al., 2021). These results indicate that the *mcr-1* gene occurs in Brazilian livestock and could contribute to the acceleration of the worldwide spread of the *mcr-1* gene and the associated MDR issue. For instance, Gelbíčová et al., (2019) found the *mcr-1* gene in *E. coli* and *K. pneumoniae* isolates in Czech Republic retail meat imported from Brazil (Gelbíčová et al., 2019). The results of this study contribute to the statistics on colistin-resistance strain, as there is a lack of information regarding Colistin resistance in microorganisms from food-producing farm animals and food products (Günaydin, Önat, & Mursaloğlu, 2024), with most studies primarily focusing on human samples.

Researchers reported in Brazil, the discovery of *E. coli* harboring *mcr-1* isolated from food and animal samples in 2016 (Organización Panamericana de la Salud & Organización Mundial de la Salud, 2016; Daza-Cardona et al. 2022), despite the fact that Brazil´s regulatory instruction in 2016 prohibited the importation and manufacture of colistin sulfate for use as a zootechnical additive (Ministério Público Federal, 2016). However, in some studies conducted in Brazil with isolates from broiler chickens and even laying hens, the sampling date is not provided, (H. P. Lopes et al., 2020; Monte et al., 2017; Saidenberg et al., 2020; Daza-Cardona et al., 2022) complicating the epidemiological assessment of whether the percentages of *mcr* gene-carrying strains have actually decreased following the ban on the use of colistin as a zoothecnical additive. It is imperative to conduct further research in different cities. It is imperative to conduct further research in different cities and municipalities in the northeastern Brazil, including the year of sample collection, as well as in production poultry.

This suggests that, that during this frame, several factors may correlate with the presence of the *mcr-1* gene. Increased research on such samples has revelated a rising percentage of the *mcr-1* gene. Additionally, this phenomenon is expected due to the dissemination of the plasmid carrying the colistin resistance gene through wastewater, environmental exposure, the global transport of animals and humans living in rural areas have also been identified as risk factors to be infected by foodborne pathogens that carry antimicrobial resistance genes (Daza-Cardona et al., 2022).

This underscores the importance of studying MDR strains from a One Health perspective to understand the dynamics of dissemination, along with their variants and origins. Measures must be implemented to mitigate the spread of strains carrying the *mcr* gene. These include testing samples from animals and food products derived from them, and companion animals that may come into direct contact with production animals. Additionally, it is critical to control wastewater and waste contaminated with hospital residues, raise awareness about the dangers of self-medication in humans, and avoid administering antibiotics to non-human animals without veterinary prescription.

As far as we know, this is the first report of the *mcr*-1 gene occurring in *E. coli* from processed food in Brazil (cheese). Other authors have found the gene in chicken meat (Monte et al. 2017; P. C. Vasconcelos et al. 2020) or in *Salmonella* spp. isolates (Rau et al., 2020; Moreno et al., 2018). The significance of these findings lies in the potential ease of transmission of *E. coli* to humans who consume these contaminated foods, whether of animal or vegetable origin. In the review by Barlaam et al., (2019), they emphasize the transmission of colistin resistance genes in foods such as raw meat and unpasteurized milk (Barlaam et al., 2019). The majority of strains isolated with acquired resistance to colistin in the food chain are *E. coli*, which is in accordance with our findings.

It is also important highlight that “artisanal” processed foods, such as the analyzed cheese samples, pose an even greater possibility of successful *mcr* gene transfer due the potential failures in the manufacturing process, where materials and inputs, along with quality controls, are often unknown.

However, the study by Barlaam et al., (2019) reports that in cheese and milk isolates in Germany, the *mcr* gene was not detected, probably because the evaluated samples were collected between 2010 and 2015 (Barlaam et al., 2019). Still, it is possible that after this period, the frequency of expression of this gene increased due to a greater possibility of dissemination by the use of polymyxins in animals. It is known that the transmission of the *mcr* gene started from domestic animals to humans through foods such as milk, meat, and eggs (Gharaibeh & Shatnawi, 2019). Additionally, the study by Barlaam et al., (2019) reported a high expression of the *mcr* gene in Brazil's chicken meat samples in 2016 (19.5%) (Barlaam et al., 2019).

The *mcr-1* has also been reported in other countries in the region, Asia, Europe, and North America (M. R. Fernandes et al., 2016). For instance, in Argentina, the *mcr*-1 gene was detected in at least one-third of 152 *E. coli* isolates recovered from poultry between 2013 and 2017 (Dominguez et al., 2017), demonstrating the gene's horizontal transmission between animals and other potential sources. According to the European Medicines Agency (EMA), in 2015 in Germany, the frequencies of the gene present in poultry and animal food were 2% and 8%, respectively (European Medicines Agency, 2016), similar to the findings of our study. In China, a retrospective study on the prevalence of the *mcr-1* gene in *E. coli* and *K. pneumoniae* isolates collected from 2011 to 2014 demonstrated the presence of the *mcr-1* gene in 78 (15%) of 525 samples of raw meat, 166 (21%) of 804 animal samples, and 16 (1%) of 1,322 samples from humans hospitalized with infections (Y.-Y. Liu et al., 2016). However, they found a lower percentage of the gene in human samples than our findings (10%). This difference could be the result of a larger number of isolates and variations in data collection for the samples. According to these findings, the *mcr-1* gene has been widely observed worldwide in *E. coli*, *K. pneumoniae*, and *Salmonella* spp. from animals, environments, and humans (Al-Tawfiq, Laxminarayan & Mendelson, 2017; WHO, 2018).

Despite that the *mcr-1* gene has been detected in Northeastern coastal water (Cordeiro-Moura et al., 2022), and mangroves (Sacramento et al., 2018), we did not find the gene in our water samples. However, as *E. coli* plays a key role in the spread of antimicrobial resistance in community settings, further investigations on water quality and safety attributes other than classic bacteria counts should be considered (Cordeiro-Moura et al., 2022). Likewise, more screenings and surveillance would be necessary to confirm the absence of *E. coli* harboring the *mcr-1* gene in this location of the Northeastern region.

In general detection of the *mcr-1* gene may be underestimated in *E. coli* strains with sensitivity to colistin, as they may contain the gene but they are not expressing it (M. R. Fernandes et al., 2016). Thus, for epidemiological purposes, regardless of the profile of the antibiogram one should carry out the detection of the *mcr* gene in

any sample. Even in isolates that show sensitive or intermediate sensitivity for colistin. In 2020, the CLSI changed the cut-off points of colistin and polymyxins B for the interpretation of Enterobacteria, *Pseudomonas aeruginosa* and *Acinetobacter* spp., which are now classified into two categories, namely, intermediate (≤ 2 µg/mL) and resistant (≥ 4 µg/mL) (Clinical and Laboratory Standards Institute, 2020a). Therefore, the cataloging of many samples that were previously classified as sensitive now be reclassified, could only be inhibited by the maximum recommended doses of these drugs, which could be a severe risk for gene dissemination into the environment. In addition, the sensitive category was eliminated because now there are not MIC values associated with a high probability of treatment success, and these higher concentrations have high nephrotoxicity (Red WHONET Argentina, 2019; Tsuji et al., 2019).

In the present study, human positive *K. pneumoniae mcr* isolates that had MIC < 0.25 µg/mL were previously classified as sensitive according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the 2019 CLSI. A possible reason for this finding may be related to non-gene expression as these microorganisms did not show the typical phenotype of resistance to polymyxins, but according to the current CLSI classification (Clinical and Laboratory Standards Institute, 2020b), they are classified as strains with intermediate sensitivity. In the research by Pillonetto et al., (2018), *E. coli* strains isolated from humans were reported in Paraná state. Most of them had MIC of 2 µg/mL (Pillonetto et al., 2018) for colistin, showing the same dynamics of microorganisms with borderline MIC or at the sensitivity limit according to the 2019 CLSI classification. Still, for the new classification, these strains have intermediate sensitivity. The three isolates carrying the *mcr-1* gene show a similar profile in the antimicrobial susceptibility test, exhibiting resistance to most cephalosporins and ciprofloxacin. However, they demonstrate sensitivity to carbapenem antibiotics, as reported in the study by Girardello et al., (2021) (Girardello et al., 2021) and resistance to ampicillin and ampicillin/sulbactam (Mota, Oliveira, & Souto, 2018).

Our identification of colistin-resistant *E. coli* strains that carry the *mcr-1* gene (MIC 4 µg/mL) shows the typical dynamics of this plasmid in microorganisms from various sources (humans, animals and food) that presented MIC greater than or equal to 4 µg/mL. Therefore, our findings support the idea that the *mcr-1* gene coming from samples of different origins could spread around the environment and thus emphasize the importance of keeping a continual epidemiological surveillance of these genes. Futures perspectives include conducting sequencing to determine the origin and identity of the plasmid.

5. CONCLUSION

Taking a One Health approach, the emergence of clinically relevant bacterial strains with plasmid-mediated transmissible resistance to colistin in human, animal, and food samples in Latin America and the Caribbean (Monte et al., 2017; Yauri-Condor et al. 2020; V. Rocha, Paiva, and Lima 2019) is an underestimated public and environmental health hazard that demands increased attention (V. Rocha, Paiva, & Lima, 2019).

The *mcr* gene, generating encodes cytoplasmic transmembrane proteins of gram-negative bacteria, generating resistance to the antibiotic colistin and most studies demonstrate that humans and animals are colonized by these commensal microorganisms (Anyanwu, Jaja, & Nwobi, 2020). In addition, the vast biodiversity and geography of the region brings back many challenges concerning the epidemiology of diseases and antimicrobial resistance (Ramon-Pardo, Sati, and Galas 2018).

The detection of the *mcr-1* gene is an essential epidemiological indicator. As far as we know, this is the first report of horizontal transfer genes for colistin resistance in the Northeastern region of the Ceará state. *E. coli* isolates from animals and food showed the typical resistance profile reported worldwide. Thus, we recommend carrying out more studies to verify the frequency of the *mcr-2* not only in the Northeastern but also throughout Brazil and the entire South American region.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ETHICAL APPROVAL

To address the ethical aspects of research involving human subjects and based on the

understanding that health studies require methodological and ethical rigor, the research was conducted in accordance with Resolution No. 466/12 of the National Health Council (CNS), which establishes the norms and guidelines for research involving human subjects.

The ethical principles of respect for individuals (autonomy and protection of vulnerable groups), beneficence, non-maleficence, and justice were upheld. Accordingly, the research was submitted to the Research Ethics Committee (CEP) of the Vale do Acaraú State University and received approval through CEP/UVA Opinion No. 4.206.357.

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Aires, C. A. M., da Conceição-Neto, O. C., Tavares e Oliveira, T. R., Dias, C. F., Montezzi, L. F., Picão, R. C., Albano, R. M., Asensi, M. D., & C.-A. A. (2017). Emergence of the plasmid-mediated *mcr-1* gene in clinical *Klebsiella pneumoniae* sequence type 392 in Brazil. *Antimicrobial Agents and Chemotherapy, 61*(7), 2–4.

Al-Tawfiq, J. A., Laxminarayan, R., & Mendelson, M. (2017). How should we respond to the emergence of plasmid-mediated colistin resistance in humans and animals? *International Journal of Infectious Diseases, 54*, 77–84. https://doi.org/10.1016/j.ijid.2016.11.415

Anyanwu, M. U., Jaja, I. F., & Nwobi, O. C. (2020). Occurrence and characteristics of mobile colistin resistance (*Mcr*) gene-containing isolates from the environment: A review. *International Journal of Environmental Research and Public Health, 17*(3), 1–38. https://doi.org/10.3390/ijerph17031028

Barbieri, N. L., Nielsen, D. W., Wannemuehler, Y., Cavender, T., Hussein, A., Yan, S. G., Logue, C. M. (2017). *Mcr-1* identified in avian pathogenic *Escherichia coli* (*APEC*). *PLoS ONE, 12*(3), 1–13. https://doi.org/10.1371/journal.pone.0172997

Barlaam, A., Parisi, A., Spinelli, E., Caruso, M., Di Taranto, P., & Normanno, G. (2019). Global emergence of colistin-resistant *Escherichia coli* in food chains and associated food safety implications: A review. *Journal of Food Protection, 82*(8), 1440–1448. https://doi.org/10.4315/0362-028X.JFP-19-116

Cavaco, L., Mordhorst, H., & Hendriksen, R. (2016). Laboratory protocol: PCR for plasmid-mediated colistin resistance genes. *DTU Food National Food Institute, 2*(October 2016), 1–15.

Clinical and Laboratory Standards Institute. (2020a). *Performance standards for antimicrobial susceptibility testing: MR01 - Polymyxin cutoff points for Enterobacterales, P. aeruginosa and Acinetobacter spp.* CLSI. (pp. 1–3).

Clinical and Laboratory Standards Institute. (2020b). *Standards for antimicrobial susceptibility testing. Performance standards for antimicrobial susceptibility testing - CLSI* (pp. 1–13).

Collignon, P., & McEwen, S. (2019). One Health—Its importance in helping to better control antimicrobial resistance. *Tropical Medicine and Infectious Disease, 4*(1), 22. https://doi.org/10.3390/tropicalmed4010022

Conceição-Neto, O. C., Aires, C. A. M., Pereira, N. F., da Silva, L. H. J., Picão, R. C., Siqueira, B. N., Albano, R. M., Asensi, M. D., & Carvalho-Assef, A. P. D. (2017). Detection of the plasmid-mediated *mcr-1* gene in clinical KPC-2-producing *Escherichia coli* isolates in Brazil. *International Journal of Antimicrobial Agents, 50*, 282–284. https://doi.org/10.1016/j.ijantimicag.2017.05.003

Cordeiro-Moura, J. R., Kraychete, G. B., Longo, L. G. de A., Corrêa, L. L., da Silva, N. M. V., Campana, E. H., … Picão, R. C. (2022). Description and comparative genomic analysis of an *mcr-1*-carrying *Escherichia coli* ST683/CC155 recovered from touristic coastal water in Northeastern Brazil. *Infection, Genetics and Evolution, 97*. https://doi.org/10.1016/j.meegid.2021.105196

Costa-Júnior, S. D., Ferreira, Y. L. A., Agreles, M. A. A., Alves, Á. E. F., Melo de Oliveira, M. B., & Cavalcanti, I. M. F. (2023). Gram-

negative bacilli carrying *mcr* gene in Brazil: A pathogen on the rise. *Brazilian Journal of Microbiology, 54*(2), 1009–1020. https://doi.org/10.1007/s42770-023-00948-w

Cyoia, P. S., Koga, V. L., Nishio, E. K., Houle, S., Dozois, C. M., Cristina, K., Kobayashi, T. (2019). Distribution of *ExPEC* virulence in *Escherichia coli* isolated from commercialized chicken carcasses. *Frontiers in Microbiology, 9*(January), 1–9. https://doi.org/10.3389/fmicb.2018.03254

Dalmolin, T., Francisco, A., Prehn, A., Lima-Morales, D. D., & Luís, A. (2018). Acquisition of the *mcr-1* gene by a high-risk clone of KPC-2-producing *Klebsiella pneumoniae* ST437/CC258, Brazil. *Diagnostic Microbiology & Infectious Disease, 90*(2), 132–133. https://doi.org/10.1016/j.diagmicrobio.2017.09.016

Daza-Cardona, E. A., Buenhombre, J., Fontenelle, R. O. dos S., & Barbosa, F. C. B. (2022). *Mcr*-mediated colistin resistance in South America, a One Health approach: A review. *Reviews in Medical Microbiology, 33*(1), e119–e136. https://doi.org/10.1097/mrm.0000000000000293

Dominguez, J. E., Figueroa Espinosa, R. A., Redondo, L. M., Cejas, D., Gutkind, G. O., Chacana, P. A., … Fernández-Miyakawa, M. E. (2017). Plasmid-mediated colistin resistance in *Escherichia coli* recovered from healthy poultry. *Revista Argentina de Microbiología, 49*(3), 297–298. https://doi.org/10.1016/j.ram.2017.02.001

European Medicines Agency. (2016). Updated advice on the use of colistin products in animals within the European Union: Development of resistance and possible impact on human and animal health. *European Medicines Agency, 44*.

Feng, S., Liang, W., Li, J., Chen, Y., Zhou, D., Liang, L., … Tian, G. B. (2022). *MCR-1*-dependent lipid remodelling compromises the viability of Gram-negative bacteria. *Emerging Microbes and Infections, 11*(1), 1236–1249. https://doi.org/10.1080/22221751.2022.2065934

Fernandes, M. R., McCulloch, J. A., Vianello, M. A., Moura, Q., Pérez-Chaparro, P. J., Esposito, F., … 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. *Antimicrobial Agents and Chemotherapy, 60*(10), 6415–6417. https://doi.org/10.1128/AAC.01325-16

Fernandes, M. R., Moura, Q., Sartori, L., Silva, K. C., Cunha, M. P., Esposito, F., … Lincopan, N. (2016). Silent dissemination of colistin-resistant *Escherichia coli* in South America could contribute to the global spread of the *mcr-1* gene. *Eurosurveillance, 21*(17), 1–6. https://doi.org/10.2807/1560-7917.ES.2016.21.17.30214

Fuentes-Castillo, D., Sellera, F. P., Goldberg, D. W., Fontana, H., Esposito, F., Cardoso, B., … Lincopan, N. (2021). Colistin-resistant *Enterobacter kobei* carrying *mcr*-9.1 and *bla* CTX-M-15 infecting a critically endangered franciscana dolphin (*Pontoporia blainvillei*), Brazil. *Transboundary and Emerging Diseases*. https://doi.org/10.1111/tbed.13980

Fuga, B., Sellera, F. P., Esposito, F., Moura, Q., Pillonetto, M., & Lincopan, N. (2024). Hybrid genome assembly of colistin-resistant *mcr-1.5*-producing *Escherichia coli* ST354 reveals phylogenomic pattern associated with urinary tract infections in Brazil. *Journal of Global Antimicrobial Resistance, 37*, 37–41. https://doi.org/10.1016/j.jgar.2024.02.017

Garza-Ramos, U., Tamayo-Legorreta, E., Arellano-Quintanilla, D. M., Rodriguez-Medina, N., Silva-Sanchez, J., Catalan-Najera, J., … Alpuche-Aranda, C. (2018). Draft genome sequence of a multidrug- and colistin-resistant *mcr-1*-producing *Escherichia coli* isolate from a swine farm in Mexico. *Genome Announcements, 6*(10), 9–10. https://doi.org/10.1128/genomeA.00102-18

Gelbíčová, T., Baráková, A., Florianová, M., Jamborová, I., Zelendová, M., Pospíšilová, L., … Karpíšková, R. (2019). Dissemination and comparison of genetic determinants of *mcr*-mediated colistin resistance in *Enterobacteriaceae* via retailed raw meat products. *Frontiers in Microbiology, 10*(December). https://doi.org/10.3389/fmicb.2019.02824

Gharaibeh, M. H., & Shatnawi, S. Q. (2019). An overview of colistin resistance, mobilized colistin resistance genes dissemination, global responses, and the alternatives to colistin: A review. *Veterinary World, 12*(11), 1735–1746.

https://doi.org/10.14202/vetworld.2019.1735-1746

Girardello, R., Piroupo, C. M., Martins, J., Maffucci, M. H., Cury, A. P., Franco, M. R. G., … Setubal, J. C. (2021). Genomic characterization of *mcr-1.1*-producing *Escherichia coli* recovered from human infections in São Paulo, Brazil. *Frontiers in Microbiology, 12*(June), 1–9. https://doi.org/10.3389/fmicb.2021.663414

Günaydin, E., Önat, K., & Mursaloğlu, P. (2024). Emergence of colistin resistance in producing *Escherichia coli* isolates from dairy cows in Türkiye. *Journal of Advances in Microbiology, 24*(12), 119–127.

Harbarth, S., Balkhy, H. H., Goossens, H., Jarlier, V., Kluytmans, J., Laxminarayan, R., … Healthcare-associated, W. (2015). Antimicrobial resistance: One world, one fight! *Antimicrobial Resistance and Infection Control, 1–15*. https://doi.org/10.1186/s13756-015-0091-2

Hussein, N. H., AL-Kadmy, I. M. S., Taha, B. M., & Hussein, J. D. (2021). Mobilized colistin resistance (*mcr*) genes from 1 to 10: A comprehensive review. *Molecular Biology Reports, 48*(3), 2897–2907. https://doi.org/10.1007/s11033-021-06307-y

Ishii, Y., Aoki, K., Endo, S., Kiyota, H., Aoyagi, T., Kaku, M., … Tateda, K. (2018). Spread of *mcr-1.5* in the community: An emerging threat. *International Journal of Antimicrobial Agents, 51*(1), 161–162. https://doi.org/10.1016/j.ijantimicag.2017.10.015

Jiménez Pearson, M. A., Galas, M., Corso, A., Hormazábal, J. C., Duarte Valderrama, C., Salgado Marcano, N., … ReLAVRA. (2019). Consenso latinoamericano para definir, categorizar y notificar patógenos multirresistentes, con resistencia extendida o panresistentes. *Revista Panamericana de Salud Pública, 43*, 8. https://doi.org/10.26633/rpsp.2019.65

Kieffer, N., Nordmann, P., Micke, A. M., Zanolli, L. M., Chaby, R., Breton, A., … Poirel, L. (2018). Genetic and functional characterization of an *MCR-3*-like enzyme-producing *Escherichia coli* isolate recovered from swine in Brazil. *Antimicrobial Agents and Chemotherapy, 62*(2). https://doi.org/10.1128/AAC.01510-17

Liu, B.-T., Li, X., Zhang, Q., Shan, H., Zou, M., & Song, F.-J. (2019). Colistin-resistant mcr-positive *Enterobacteriaceae* in fresh vegetables, an increasing infectious threat in China. *International Journal of Antimicrobial Agents, 54*(1), 89–94. https://doi.org/10.1016/j.ijantimicag.2019.04.013

Liu, M., Wu, J., Zhao, J., Xi, Y., Jin, Y., Yang, H., … Duan, G. (2024). Global epidemiology and genetic diversity of mcr-positive *Klebsiella pneumoniae*: A systematic review and genomic analysis. *Environmental Research, 259*(100), 119516. https://doi.org/10.1016/j.envres.2024.119516

Liu, Y.-Y., Wang, Y., Walsh, T. R., Yi, L.-X., Zhang, R., Spencer, J., … 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. *The Lancet Infectious Diseases, 16*(2), 161–168. https://doi.org/10.1016/S1473-3099(15)00424-7

Lopes, H. P., Costa, G. A., Pinto, A. C. L. Q., Machado, L. S., Cunha, N. C., Nascimento, E. R., … Abreu, D. L. C. (2020). Detection of the mcr-1 gene in enteropathogenic *Escherichia coli* (EPEC) and shigatoxigenic *E. coli* (STEC) strains isolated from broilers. *Pesquisa Veterinaria Brasileira, 40*(3), 165–169. https://doi.org/10.1590/1678-5150-PVB-5983

Lopes, R., Furlan, J. P. R., dos Santos, L. D. R., Gallo, I. F. L., & Stehling, E. G. (2021). Colistin-resistant mcr-1-positive *Escherichia coli* ST131-H22 carrying blaCTX–M–15 and qnrB19 in agricultural soil. *Frontiers in Microbiology, 12*(April), 1–12. https://doi.org/10.3389/fmicb.2021.659900

McEwen, S. A., & Collignon, P. J. (2018). Antimicrobial resistance: A One Health colloquium. *Microbiology Spectrum, 6*(2), 1–26. https://doi.org/10.1128/microbiolspec.ARBA-0009-2017.Correspondence

Medina, J. (2017). Actualización acerca de colistina (polimixina E): Aspectos clínicos, PK/PD y equivalencias. *Revista Medica Del Uruguay, 33*(3), 195–206. https://doi.org/10.29193/rmu.33.3.5

Ministério Público Federal. (2016). O COMUNICADO DE RISCO no 01/2016 – GVIMS/GGTES/ANVISA da ANVISA alerta sobre a descoberta de uma nova cepa da

bactéria *Escherichia coli* em amostras de pacientes atendidos nos hospitais no Brasil, que apresentaram resistência ao antibiótico colistina (Poli) (pp. 1–27).

Monte, D. F., Mem, A., Fernandes, M. R., Cerdeira, L., Esposito, F., Galvão, J. A., … Landgraf, M. (2017). Chicken meat as a reservoir of colistin-resistant *Escherichia coli* strains carrying mcr-1 genes in South America. *Antimicrobial Agents and Chemotherapy, 61*(5), 1–12. https://doi.org/10.1128/AAC.02718-16

Moreno, L. Z., Gomes, V. T. M., Moreira, J., Oliveira, H. De, Peres, B. P., Silva, A. P. S., … Moreno, A. M. (2018). First report of mcr-1-harboring *Salmonella enterica* serovar Schwarzengrund isolated from poultry meat in Brazil. *Diagnostic Microbiology & Infectious Disease*. https://doi.org/10.1016/j.diagmicrobio.2018.10.016

Mota, F. S. da, Oliveira, H. A. de, & Souto, R. C. F. (2018). Profile and prevalence of antimicrobial resistance of negative-Gram bacteria isolated from intensive care patients. *Revista Brasileira de Análises Clínicas, 50*(3). https://doi.org/10.21877/2448-3877.201800740

Nesporova, K., Jamborova, I., Valcek, A., Medvecky, M., Literak, I., & Dolejska, M. (2019). Various conjugative plasmids carrying the mcr-5 gene in *Escherichia coli* isolates from healthy chickens in Paraguay. *Journal of Antimicrobial Chemotherapy*, 3394–3397. https://doi.org/10.1093/jac/dkz317

Organización Panamericana de la Salud, & Organización Mundial de la Salud. (2016). Enterobacterias con resistencia transferible a colistina, implicaciones para la salud pública en las Américas. *Alerta Epidemiológica OPS/OMS*, 1–5. Retrieved from https://www.paho.org/hq/dmdocuments/2016/2016-jun-10-alerta-epi-enterob-resist.pdf

Owolabi, S. L., & Azeez, I. A. (2020). Antimicrobial resistance profile of *Escherichia coli* isolates from different clinical samples in Abeokuta, Ogun State. *South Asian Journal of Research in Microbiology, 7*(1), 12–18. https://doi.org/10.9734/sajrm/2020/v7i130160

Paiva, Y., Nagano, D. S., Luis, A., Cotia, F., Cristina, R., Martins, R., … Costa, S. F. (2021). Colistin-resistant *Escherichia coli* belonging to different sequence types: Genetic characterization of isolates responsible for colonization, community- and healthcare-acquired infections. *Revista Do Instituto de Medicina Tropical de São Paulo, 63*(April), 1–6.

Palmeira, J. D., Ferreira, H., Madec, J. Y., & Haenni, M. (2018). Draft genome of a ST443 mcr-1- and blaCTX-M-2-carrying *Escherichia coli* from cattle in Brazil. *Journal of Global Antimicrobial Resistance, 13*, 269–270. https://doi.org/10.1016/j.jgar.2018.05.010

Papa-Ezdra, R., Diaz, F. G., Vieytes, M., García-Fulgueiras, V., Caiata, L., Ávila, P., … Vignoli, R. (2019). First three *Escherichia coli* isolates harboring mcr-1 in Uruguay. *Journal of Global Antimicrobial Resistance*. https://doi.org/10.1016/j.jgar.2019.07.016

Perdigão Neto, L. V., Corscadden, L., Martins, R., Nagano, D., Cunha, M. P. V., Neves, P. R., … Costa, S. F. (2019). Simultaneous colonization by *Escherichia coli* and *Klebsiella pneumoniae* harboring mcr-1 in Brazil. *Infection, 47*(4), 1–4. https://doi.org/10.1007/s15010-019-01309-2

Pillonetto, M., Mazzetti, A., Becker, G. N., Siebra, C. A., Arend, L. N. V. S., & Barth, A. L. (2018). Low level of polymyxin resistance among non-clonal mcr-1-positive *Escherichia coli* from human sources in Brazil. *Diagnostic Microbiology & Infectious Disease*, 10. https://doi.org/10.1016/j.diagmicrobio.2018.08.009

Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: A global multifaceted phenomenon. *Pathogens and Global Health, 109*(7), 309–318. https://doi.org/10.1179/2047773215Y.0000000030

Ramon-Pardo, P., Sati, H., & Galas, M. (2018). “One health” approach in the actions to address antimicrobial resistance from a Latin American standpoint. *Revista Peruana de Medicina Experimental y Salud Publica, 35*(1), 103–109. https://doi.org/10.17843/rpmesp.2018.351.3605

Rau, R. B., De Lima-Morales, D., Wink, P. L., Ribeiro, A. R., & Barth, A. L. (2020). *Salmonella enterica* mcr-1 Positive from food in Brazil: Detection and characterization. *Foodborne Pathogens*

and Disease, 17(3), 202–208. https://doi.org/10.1089/fpd.2019.2700

Red WHONET Argentina. (2019). *Protocolo De Trabajo Red WHONET Argentina*. XIX Taller WHONET-Argentina, 1–56. Retrieved from http://antimicrobianos.com.ar/ATB/wp-content/uploads/2014/10/Protocolo-WHONET-consensuado-2017-final.pdf

Rocha, C., Reynolds, N. D., & Simons, M. P. (2015). Emerging antibiotic resistance: A global threat and critical healthcare problem. *Revista Peruana de Medicina Experimental y Salud Publica, 32*(1), 139–145. https://doi.org/10.17843/rpmesp.2015.321.1586

Rocha, I. V., Alberto, C., Campos, T. D. L., Rezende, A. M., Leal, N. C., Fernanda, C., … Xavier, D. E. (2017). Ciprofloxacin-resistant and extended-spectrum β-lactamase-producing *Escherichia coli* ST410 strain carrying the mcr-1 gene associated with bloodstream infection. *International Journal of Antimicrobial Agents, 49*(5), 655–656. https://doi.org/10.1016/j.ijantimicag.2017.03.001

Rocha, V., Paiva, M. C., & Lima, W. G. (2019). Plasmid-mediated colistin resistance in Latin America and the Caribbean: A systematic review. *Travel Medicine and Infectious Disease, 31*(February), 101459. https://doi.org/10.1016/j.tmaid.2019.07.015

Saavedra, S. Y., Arévalo, A., Ovalle, M. V., Montaño, L. A., Hidalgo, A. M., Duarte, C., & Beltrán, M. (2016). Alerta por la primera detección de mcr-1 gen de resistencia a colistina en aislamientos de *Salmonella enterica* serovar Typhimurium y *Escherichia coli* de origen humano en Colombia. *Instituto Nacional de Salud*, 4–8. Retrieved from https://www.ins.gov.co/buscador-eventos/Informacin%20de%20laboratorio/Alerta%20Colombia%20mcr1%20Salmonella%20y%20E.%20coli.pdf

Sacramento, A. G., Fernandes, M. R., Sellera, F. P., Muñoz, M. E., Vivas, R., Dolabella, S. S., & Lincopan, N. (2018). Genomic analysis of MCR-1 and CTX-M-8 co-producing *Escherichia coli* ST58 isolated from a polluted mangrove ecosystem in Brazil. *Journal of Global Antimicrobial Resistance, 15*, 288–289. https://doi.org/10.1016/j.jgar.2018.10.024

Saidenberg, A. B. S., Stegger, M., Price, L. B., Johannesen, T. B., Aziz, M., Cunha, M. P. V., … Knöbl, T. (2020). mcr-positive *Escherichia coli* ST131-H22 from poultry in Brazil. *Emerging Infectious Diseases, 26*(8), 459–461. https://doi.org/10.1016/B978-0-12-386454-3.01006-X

Shad, A. A. (2018). MCR-1 colistin resistance in *Escherichia coli* wildlife: A continental mini-review. *Journal of Drug Metabolism & Toxicology, 09*(03), 9–11. https://doi.org/10.4172/2157-7609.1000243

Touati, M., Hadjadj, L., Berrazeg, M., Baron, S., & Rolain, J. M. (2019). Emergence of *Escherichia coli* harboring mcr-1 and mcr-3 gene in North West Algerian farmlands. *Journal of Global Antimicrobial Resistance, 21*, 132–137. https://doi.org/10.1016/j.jgar.2019.10.001

Tsuji, B. T., Pogue, J. M., Zavascki, A. P., Paul, M., Daikos, G. L., Forrest, A., … Kaye, K. S. (2019). International consensus guidelines for the optimal use of the polymyxins: Endorsed by the American College of Clinical Pharmacy (ACCP), European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Infectious Diseases Society of America (IDS). *Pharmacotherapy, 39*(1), 10–39. https://doi.org/10.1002/phar.2209

Ugarte Silva, R. G., Olivo López, J. M., Corso, A., Pasteran, F., Albornoz, E., & Sahuanay Blácido, Z. P. (2018). Resistencia a colistín mediado por el gen mcr-1 identificado en cepas de *Escherichia coli* y *Klebsiella pneumoniae*: Primeros reportes en el Perú. *Anales de La Facultad de Medicina, 79*(3), 213. https://doi.org/10.15381/anales.v79i3.153113

Vasconcelos, I., Silva, S., Alberto, C., Fernanda, C., Vidal, D. L., Cintra, N., & Elias, D. (2020). Diverse and emerging molecular mechanisms award polymyxins resistance to *Enterobacteriaceae* clinical isolates from a tertiary hospital of Recife, Brazil. *Infection, Genetics and Evolution, 85*(August), 104584. https://doi.org/10.1016/j.meegid.2020.104584

Vasconcelos, P. C., Leite, E. L., Araújo, W. J., Silva, N. M. V, Saraiva, M. M. S., Santos, L., … Oliveira, C. J. B. (2020). Draft genome sequence of mcr-1-mediated colistin-resistant *Escherichia coli* ST359

from chicken carcasses in Northeastern Brazil. *Journal of Global Antimicrobial Resistance, 23*, 135–136. https://doi.org/10.1016/j.jgar.2020.08.016

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*(4), 1–8. https://doi.org/10.1371/journal.pone.0195281

World Health Organization (WHO). (2018). *The detection and reporting of colistin resistance*. WHO/WSI/AM, 1–17.

Xavier, B. B., Lammens, C., Ruhal, R., Butaye, P., & Goossens, H. (2016). Identification of a novel plasmid-mediated colistin-resistance gene, mcr-2, in *Escherichia coli*, Belgium, June. *Eurosurveillance*, (June), 6–11.

Yang, Y. H., & Buttery, J. (2018). Antimicrobial resistance: A global one-health problem for all ages. *World Journal of Pediatrics*, 2–3. https://doi.org/10.1007/s12519-018-0194-y

Yauri-Condor, K., Zavaleta Apestegui, M., Sevilla-Andrade, C. R., Piscoya Sara, J., Villoslado Espinoza, C., Vicente Taboada, W., & Gonzales-Escalante, E. (2020). Extended-spectrum beta-lactamase-producing *Enterobacterales* carrying the mcr-1 gene in Lima, Peru. *Revista Peruana de Medicina Experimental y Salud Publica, 37*(4), 711–715. https://doi.org/10.17843/rpmesp.2020.374.5832

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/128736