Original Research Article

Detection of Plasmid-mediated Colistin Resistance Genes among *Escherichia coli* and *Klebsiella pneumoniae*Isolated fromPoultry, Food and Human Clinical Samples: A One Health Approach for Antimicrobial Resistance in Brazil

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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 polymerasechain 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. 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, and Pantosti 2015; Shad 2018; B.-T. Liu et al. 2019). 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 and 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 and 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 and 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, and 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, and 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 and 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. MATERIAL 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 isolates from human clinical samples were collected in 2015, those from water and food samples between 2017 and 2018, and those from poultry in 2020. 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.

2.4Polymerase 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) (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, and 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).

2.5Minimum 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.

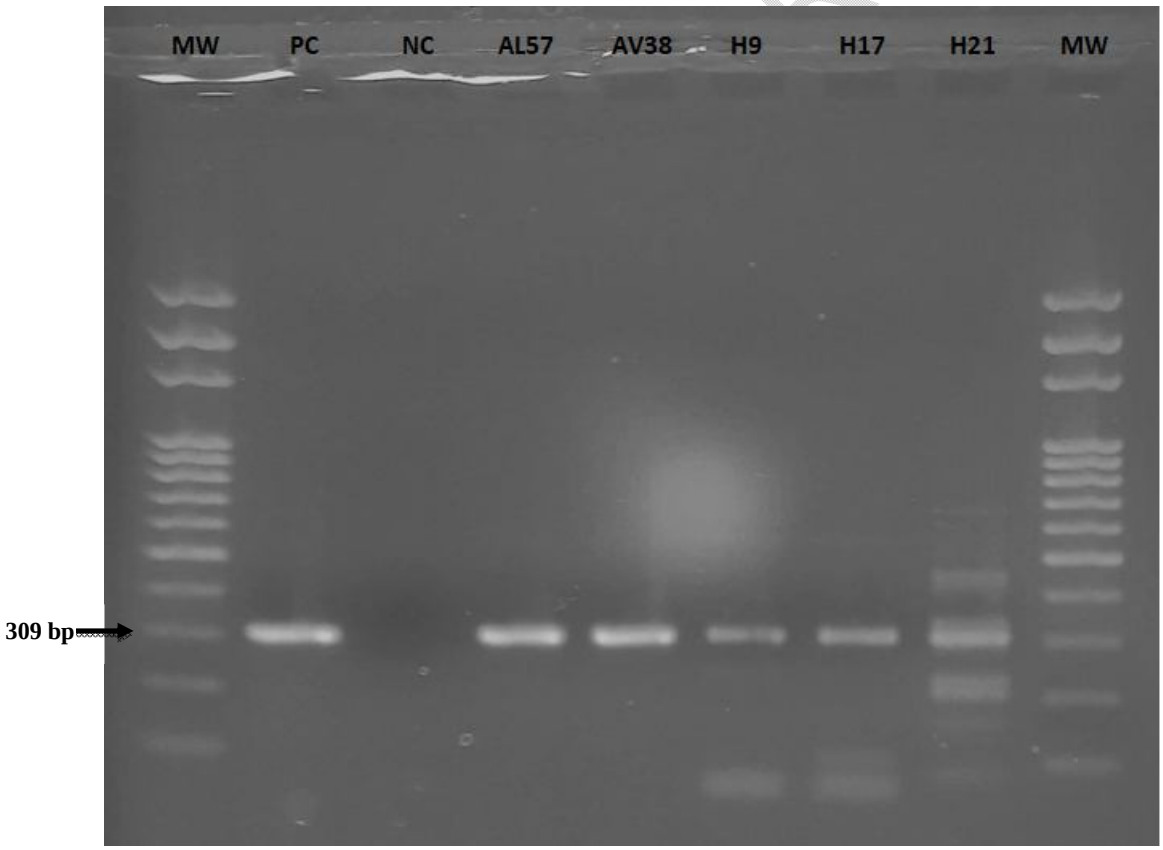

Fig. 1. Amplification products obtained by PCR performed for the detection of the *mcr-1*

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.

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*

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).

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

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).

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.

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, and 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, and Galas 2018). 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 CAM, da Conceição-Neto OC, Tavares e Oliveira TR, Dias CF, Montezzi LF, Picão RC, Albano RM, Asensi MD 2017) where high prevalence rates are reported for *K. pneumoniae* have been observed in secretions (Mota, Oliveira, and 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; 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 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).

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 and 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, and 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. pneumoniaemcr* 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 Giardello et al. (2021) (Girardello et al. 2021) and resistance to ampicillin and ampicillin/sulbactam (Mota, Oliveira, and 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.

4. 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, and 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, and 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.

ETHICAL APPROVAL

To address to the ethical aspects in research involving human, the study was summited to the research ethics committee (cep) of the state university of vale do acaraú and received approval under 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.

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