ENVIRONMENTAL MICROBIOLOGY - REVIEW

Gram-negative bacilli carrying *mcr* gene in Brazil: a pathogen on the rise

Sérgio Dias Costa-Júnior[1] · Ylanna Larissa Alves Ferreira[2] · Maria Anndressa Alves Agreles[2] ·
Álisson Emannuel Franco Alves[1] · Maria Betânia Melo de Oliveira[3] · Isabella Macário Ferro Cavalcanti[2,4]

Abstract

The incidence of infections caused by resistant Gram-negative pathogens has become a critical factor in public health due to the limitation of therapeutic options for the control of infections caused, especially, by *Enterobacteriaceae* (*Escherichia coli* and *Klebsiella pneumoniae*), *Pseudomonas* spp., and *Acinetobacter* spp. Thus, given the increase in resistant pathogens and the reduction of therapeutic options, polymyxins were reintroduced into the clinic. As the last treatment option, polymyxins were regarded as the therapeutic key, since they were one of the few classes of antimicrobials that had activity against multidrug-resistant Gram-negative bacilli. Nonetheless, over the years, the frequent use of this antimicrobial has led to reports of resistance cases. In 2015, *mcr* (*mobile colistin resistanc*e), a colistin resistance gene, was described in China. Due to its location on carrier plasmids, this gene is characterized by rapid spread through conjugation. It has thus been classified as a rising threat to public health worldwide. In conclusion, based on several reports that show the emergence of *mcr* in different regional and climatic contexts and species of isolates, this work aims to review the literature on the incidence of the *mcr* gene in Brazil in different regions, types of samples identified, species of isolates, and type of carrier plasmid.

Keywords Antimicrobial resistance (AMR) · Polymyxins · Colistin resistance gene · Public health

Introduction

Recently, antimicrobial resistance (AMR) has become a critical factor in public health due to the reduction in therapeutic options associated with increased morbidity and mortality rates [1, 2]. First, with the development and commercialization of antimicrobials, along with the increase in the prevalence rates of bacterial resistance, the scientific community has focused on Gram-positive pathogens [3–5]. Thus, the change in the susceptibility pattern of pathogens and the multidrug resistance (MDR) presented by Gram-negative bacilli has become a threat to the medical and scientific community worldwide [5]. Indeed, between 2000 and 2010, approximately 70 billion doses of antibiotics were administered globally and 50% of doses are used incorrectly or unnecessarily, thus facilitating the development of AMR [1, 6].

AMR is divided into two types: (i) innate resistance and (ii) acquired resistance. Intrinsic or innate resistance is characterized by the particularities of each microorganism and depends on its biological characteristics. Furthermore, acquired resistance can result from the acquisition of resistance genes, mutation of chromosomal DNA, or both mechanisms [7]. Due to the emergence of resistant pathogens over the years, new classes of drugs have been identified. In the last 20 years, two new classes of antibiotics, lipopeptides and oxazolidinones, have been classified and approved for use. However, the rapid ability of bacteria to acquire resistance

✉ Isabella Macário Ferro Cavalcanti
   isabella.cavalcanti@ufpe.br

1   Department of Physiology and Pathology, Federal University of Paraíba (UFPB), 58.051-900, João Pessoa, Brazil

2   Keizo Asami Institute, Federal University of Pernambuco (iLIKA/UFPE), 50.670-901, Recife, Brazil

3   Department of Bio of Biochemistry, Center for Biosciences, Federal University of Pernambuco (UFPE), Av. Moraes Rego S/N, Recife, PE, Brazil

4   Microbiology and Immunology Laboratory, Academic Center of Vitória, Federal University of Pernambuco (CAV/UFPE), Centro Acadêmico de Vitória, Rua Do Alto Do Reservatório S/N, Bela Vista, Vitória de Santo Antão, PE 55608-680, Brazil

to these new classes made them ineffective against some bacterial strains [8].

According to the World Health Organization (WHO), the lack of treatment options for multidrug-resistant Gram-negative bacilli (MDR-GNB), including carbapenem-resistant, such as *Escherichia coli* and *Klebsiella pneumoniae*, *Pseudomonas* spp., and *Acinetobacter* spp., results in serious infections that can lead to public health problems [9]. The irrational use of carbapenems was due to the emergence of cephalosporinases, which in turn resulted in carbapenem resistance [3, 10]. Therefore, with the increase in carbapenem-resistant pathogens, polymyxins have been reintroduced into the clinic as one of the last options for the treatment of MDR-GNB [5, 10, 11].

Polymyxins were first discovered in 1947, but in the 1970s, they were found to have high nephrotoxic potential after their incorporation into clinical practice [12]. After the arising of frequent cases of patients with renal dysfunction and the need for safer drugs, the use of polymyxins is now reduced, requiring the use of smaller doses for this antibiotic as well as preferential use of PMB over it, or its co-administration with antioxidants [13]. However, in an era of MDR bacteria, old antimicrobials have re-emerged as treatment options, being, in some cases, the only ones with activity against MDR-GNB [13, 14].

Ho et al. [15] in 2010 described polymyxins as a key therapeutic for infections caused by pathogens resistant to carbapenems. However, with the rise in the use of these drugs, an increase in cases of resistance to these lipopeptides has been noted [16]. In 2015, in China, a transferable plasmid-mediated colistin resistance gene, *mcr-1*, was identified, whose gene product, the MCR-1 protein, modifies the lipid A component of LPS, inhibiting the binding of polymyxins to target [17, 18].

It has been shown that the production of MCR-1 in pathogens such as *E. coli* leads to an increase in the minimum inhibitory concentrations (MICs) of polymyxins by up to eight times; therefore, even without additional resistance mechanisms, the production of this enzyme is sufficient to develop resistance to polymyxins [16]. In addition, the location of the *mcr* gene in plasmids makes it favorable for rapid propagation by conjugation, especially in *E. coli* isolates [17, 19].

Initial reports of the presence of *mcr-1* in Brazil were based on 16 samples of *E. coli* isolated from poultry and swine. Subsequently, the first case of clinical isolates and environmental samples carrying *mcr-1* has been reported in 2016 [20, 21]. Nowadays, there are several reports of isolates that produce MCR-1 related to nosocomial infections across the country [22]. Thus, the present study aims to understand, through a narrative literature review, the variants present in reports of the incidence of Gram-negative bacilli carrying the *mcr* gene in Brazil.

Characteristics of Gram-negative bacilli

Through the action of the virulence factors, bacteria use genes to carry out their infection and resist host defenses [23]. These factors are classified according to their mechanism and/or function, for example, (a) membrane proteins, which play a fundamental role in host adhesion, colonization, and invasion; (b) toxin-secreting proteins capable of modifying the host cell environment and are responsible for some host cell-bacteria interactions; (c) components of the outer membrane, such as lipopolysaccharide (LPS) that can protect against lysis mediated by the complement system; and (d) ability to form biofilms, which are bacterial aggregates contained in extracellular matrices of polysaccharides, proteins, enzymes, and nucleic acids. This matrix confers some resistance to bacteria, restricting the action of antibiotics, reducing the growth rate, and even neutralizing the immune system. Therefore, this last resistance factor associated with highlighted bacterial resistance mechanisms synergistically contributes to the wide dissemination of MDR-GNB strains [24, 25].

Gram-negative pathogens have genetic mutations and acquire mobile genetic elements that confer resistance mechanisms. Due to this, they are among the most common pathogens in community and hospital infections since they have a high transmission rate [24–26]. Furthermore, resistance genes have become a serious public health issue worldwide by compromising the effects of antimicrobials, especially β-lactams [15, 22].

As highlighted by Mota et al. [27], Enterobacterales and Gram-negative non-fermenting bacilli (GNNFB) are the most common pathogens associated with nosocomial infections. The author reported that, considering the isolated microorganisms, *K. pneumoniae*, followed by *E. coli*, *A. baumannii*, and *P. aeruginosa*, showed the highest prevalence in an intensive care unit (ICU) located in the Midwest region of Brazil. Additionally, the profile of bacterial resistance of these isolates to antimicrobials was analyzed and a significant resistance to β-lactams, such as penicillins (56.2%), second-generation cephalosporins (57.9%), third-generation cephalosporins (51.9%) and carbapenems (46.1%), and the class of antimicrobials quinolones (88.8%), was observed. In relation to GNNFB, it was realized that for *A. baumannii* the resistance could be between 80 and 100% for the β-lactams cefepime and meropenem, as well as for the fluoroquinolone ciprofloxacin, and from 50 to 70% for β-lactams the imipenem, piperacillin associated with beta-lactamase inhibitor tazobactam and ceftazidime, for the cefamicin, cefoxitin, and for the aminoglycoside gentamicin. *P. aeruginosa* isolates showed a lower rate of resistance in general when compared to other bacterial species, ranging from 30 to 40% for β-lactams cefoxitin, cefuroxime, imipenem, and meropenem [27].

Resistance mechanism in Gram-negative bacilli

The majority of antimicrobials are naturally produced, and co-resident bacteria develop a natural resistance mechanism known as intrinsic resistance [28]. After establishing itself in the genome of a microorganism's genus or species, innate resistance will spread through subsequent generations. There are several causes for this characteristic, such as lack of affinity of the drug for the target, inaccessibility to the bacterial cell interior, or extrusion of the drug by active exporters and efflux pumps, yet the innate production of enzymes that inactivate the drug [29, 30].

Acquired resistance is defined as a process in which the bacteria that originally presented susceptibility to the drug become resistant. The development of this resistance is the result of gene mutations transferred from the parent cell to the daughter cell or through gene exchange mechanisms [28]. Clinically, acquired resistance is more significant due to the high spread of resistance genes [29]. This transfer can be performed by plasmids, which can carry resistance genes to multiple classes of antibiotics and integrate into the bacterial genome [31].

The mechanism of transfer of genetic material includes conjugation, transduction, and transformation [32]. The gene transfer through conjugation occurs through the contact between the surfaces of the donor and the receptor cells with the transfer of the plasmid carrying the resistance gene. This process has been widely reported in Gram-negative pathogens [7].

The production of antimicrobial-modifying enzymes is another resistance mechanism that can be mediated by plasmids. Resistance of *Pseudomonas aeruginosa* isolates to fosfomycin is one of the examples of this type of resistance reported in the clinic [7]. Chromosomal mutations that occur in incorrect DNA repair or chromosomal replication have also been a widely reported bacterial mean of acquiring resistance, as well as the expression of efflux systems, which is responsible for the resistance to fluoroquinolones present in several Gram-negative isolates, for example [7, 32, 47].

As a result of multiple resistance mechanisms coexisting within a pathogen, a MDR phenotype has become common among isolates associated with nosocomial infections [22]. In 2011, terminologies were created to standardize and describe the profiles of broadly resistant microorganisms, such as MDR (multidrug-resistant)—resistant to 3 or more classes of antimicrobials; XDR (extensively drug-resistant)—microorganisms that remain susceptible to at least one or two antibiotics belonging to two different classes; PDR (pandrug-resistant)—proven resistance in all categories of antimicrobials tested. In this way, the increase in the detection rate of pathogens with widespread resistance MDR, XDR, and PDR is attributed to a combination of microbial features, uncontrolled use of antibiotics, and dissemination of resistance genes [30, 33].

The alarming lack of effective antimicrobial agents to combat pathogens with a wide spectrum of resistance in recent years has led to the reintroduction of polymyxins B and E, two antibiotics from the 1960s that had promising in vitro activity against resistant pathogens, but their use was limited due to their high incidence of nephrotoxicity and neurotoxicity [34, 35]. The intense use of polymyxins for the absence of equally effective antibiotics consequently led to the identification of some strains resistant to these antibiotics not long after their return, even though this resistance was chromosomal, and therefore there was no risk of rapid distribution and dissemination [36]. However, the whole scenario of the use of polymyxins is incisively threatened with the first report of plasmid resistance, attributed to the new gene *mcr-1* [37].

Polymyxins and the plasmid resistance gene

It is essential for antibiotics to penetrate bacterial amphiphilic barriers, allowing the active ingredient to enter the cell. Cyclic antibiotics have the ability to interact with bacterial membranes. In this context, the constituent compounds of this class have been used in the front line against infections caused by MDR Gram-negative isolates [38, 39].

Polymyxin was obtained from the microorganism *Paenibacillus polymyxa* [40]. Its chemical structure is composed of a decapeptide core that contains an intramolecular cycle of heptapeptides linked by amide-type bonds between the amino group of the side chain of the diaminobutyric acid (Dab) residue at position 4 and the carboxyl group on the C-threonine residue terminal. Another important structural feature of the polymyxin chain is the presence of five non-proteinogenic Dab residues, which are positively charged at physiological pH, hydrophobic residues, and an N-terminal acyl group. This class of antimicrobials is formed by a group of five compounds (A, B, C, D, and E), which differ in terms of amino acid sequences and fatty acid side chains [41]. The chemical structure of polymyxins B and E (colistin) differs by the presence of an amino acid D-Leucine in the colistin molecule, and D-phenylalanine in the polymyxin B molecule [42].

Polymyxins act by binding to lipopolysaccharide (LPS) and phospholipid molecules found in the outer membrane of gram-negative bacteria. This process results in a change in the cell wall of the microorganism, leading to extravasation of intracellular content and, consequently, to the death of the bacteria [43]. Due to the electrostatic interaction between antimicrobial molecules with the phosphate group of lipid

A of the bacterial membrane, polymyxins have a greater affinity for bacterial LPS when compared to magnesium and calcium cations, which in turn stabilize LPS [17]. This interaction causes a shift in calcium and magnesium ions, thus allowing the binding of the active principle to the membrane of the microorganism, resulting in a derangement in the membrane and, later, in an increase in the absorption of the antibiotic, which leads to osmotic imbalance, causing cell death [44].

Among the other mechanisms of action of polymyxins are the inhibition of enzymes essential for cellular respiration in bacteria, such as NADH-quinone oxidoreductase type II [45]. However, it has been reported that polymyxin susceptibility has decreased as well as an increase in infections caused by polymyxin-resistant Gram-negative pathogens. Polymyxin resistance was mainly observed in isolates of *K. pneumoniae* and *E. coli*. Thus, the clinical efficacy of this class of antimicrobials has become limited when used as monotherapy [22, 46].

The mechanism by which Gram-negative pathogens become resistant to polymyxins involves a chemical modification of the bacterial outer membrane [47]. These modifications are mediated by specific chromosomal mutations in the genes that control the two components system PhoP/Q and PmrA/B, such as the recently discovered CrrAB, which positively regulates the PmrAB system. CrrAB is responsible for the genes *crrB* that encodes a sensor kinase and *crrA* that encodes a regulatory protein and the modulator gene *crrC* regulators of the *pbgP* operon. Moreover, *mgrB* gene inhibits the PhoPQ system. This gene encodes a small transmembrane lipoprotein that is responsible for the negative regulation of the PhoPQ system. Since the two systems regulate resistance by altering the cationic charge and the ionic pattern of the outer membrane, changes in their function can lead to a decrease in the anionic charge and, consequently, prevent the electrostatic interaction of the bacterial cell with drug molecules [48–50].

Gene regulation results in the modification of LPS that leads to resistance. Gene regulation is mediated by two-component enzyme systems (TCS) that usually regulate resistance and virulence factors [49]. The inactivation of TCS regulator MgrB by non-sensing mutation, premature spot-codon, or gene interruption by insertion sequences, as well as change of the histidine kinase sensor CrrB resulting in PmrA/B over-expression, have been associated with polymyxin resistance in *Klebsiella.* Additionally, although the function of CrrAB has not yet been fully understood, it has been described that, when dysfunctional, CrrAB may also modify lipid A through the activation of a glycosyltransferase-like protein [49, 50]. Furthermore, the action of efflux pump systems, changes in protein concentrations of the outer membrane (porins), and thickening of the polysaccharide capsule are also extremely important for the development of pathogen resistance to

polymyxins among the constituents of the order Enterobacterales [38].

In addition to resistance mediated by mutations involving TCSs, the mechanism encoded by a plasmid localization gene, *mcr-1*, described in China in 2015, has become the leading cause of polymyxin resistance [17]. There are two types of chemical changes in the outer membrane of Gram-Negative bacilli that lead to resistance to polymyxins, one of the main ones is the addition of 4-amino-4-deoxy-L-arabinose (L-Ara4N) in the lipid A component of LPS, reducing membrane antimicrobial activity. Another way is the addition of phosphoethanolamine (PEA) to the 1-(4′)-phosphate group of lipid A, promoting intrinsic resistance to polymyxins [50].

MCR-1 is described as lipase A-40-PEA-transferase. This family of alkaline phosphatases catalyzes the binding of phosphatidylethanolamine (PEA) to LPS lipid A and consequently leads to colistin resistance [51, 52]. Thus, transferable polymyxin resistance is related to the addition of PEA to LPS lipid A by MCR-1 [48–52]. Furthermore, the location of the *mcr* gene on plasmids favors rapid propagation by conjugation, especially in *E. coli* isolates (Fig. 1). The presence of this gene in a variety of plasmids highlights its ability to spread (Table 1) [17, 19, 52–89].

Jeannot et al. [62] highlighted the interspecies transfer of mobile genes as a factor responsible for the progressive spread of resistance genes. Additionally, the intrinsic mechanism and rates of polymyxin resistance in *Enterobacteriaceae* and isolates of *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* was observed until 2017. In their study, Jeannot et al. [635] also confirmed that *mcr* genes are located in the transferable plasmid, which, in turn, represents a high propagation factor by conjugation.

According to the studies present on the NCBI platform, ten variants of the *mcr* gene have been described, so far, in several species of microorganisms [17, 19, 54–61]. The *mcr-1* gene has 13 variants, most of which have been reported in *E. coli* and *K. pneumoniae* isolates [63, 64]. The variants already described can be found in different classes of plasmids, characterizing a diversity of gene reservoirs. IncI2, IncHI2, and IncX4 are described as the main groups of plasmids responsible for carrying resistance genes. There are many studies describing the spread of colistin resistance related to gene variants, sites, the types of samples analyzed, the species of isolates, and the type of carrier plasmids (Table 2) [17, 21, 30, 34, 55, 60].

Incidence of the *mcr* gene in samples of clinical isolates in Brazil

In Brazil, the first Gram-negative bacteria with polymyxin resistance were identified in *E. coli* isolates [65, 66]. Cases of colistin resistance in clinical isolates in humans began
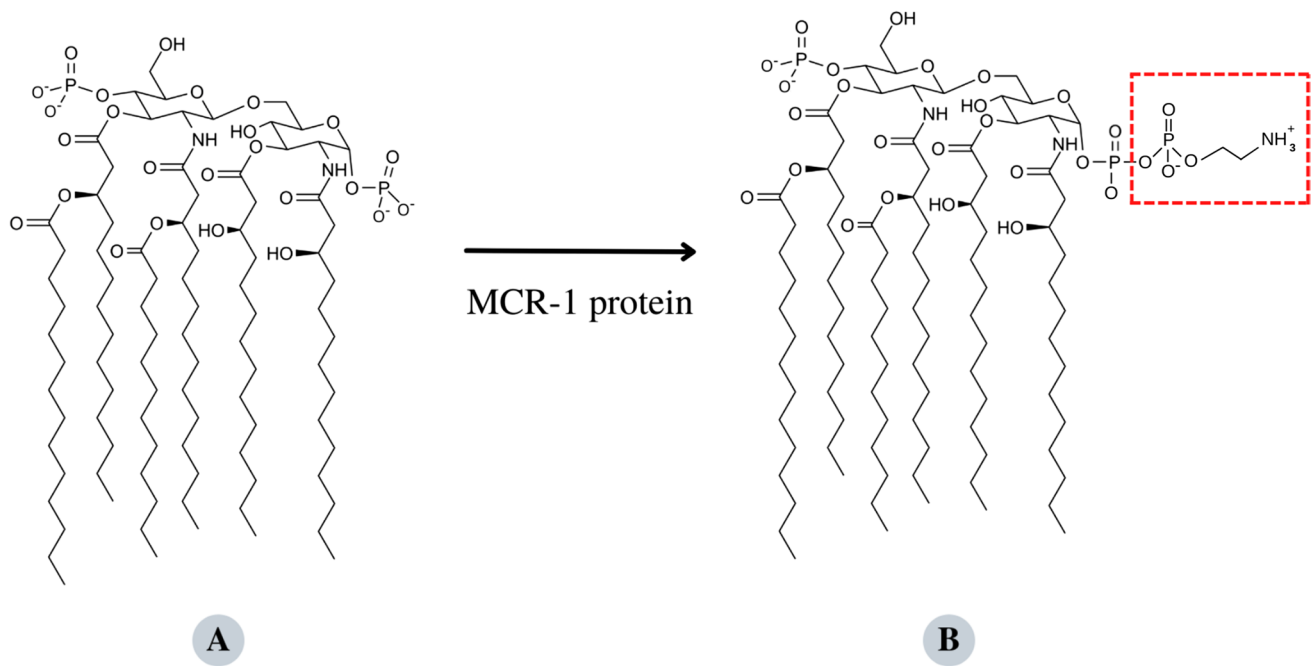
Fig. 1 Representation of MCR-1 protein activity. (**A**) Original structure of lipid A found in Gram-negative bacteria; (**B**) modification of this molecule after the action of the protein encoded by the *mcr-1* gene, characterized by the addition of phosphoethanolamine to the 1-(4′)-phosphate group of lipid A

Table 1 *mcr* gene variants involved in polymyxin resistance, year, location, plasmid, and origin of the biological sample

| *mcr* gene	Species	Genes of β-lactamases	MIC of colistin (mg/L)	Origin	Year*	Country	References
mcr-1	*E. coli*	ND**	8	Animal, human, and Food	2015	China	[17]
mcr-2	*E. coli*	ND**	4–8	Animal	2011–2012	Belgium	[19]
mcr-3	*E. coli*	bla_{OXA-1}	8	Animal	2015	China	[54]
mcr-4	*E. coli* and *Salmonella*	ND**	8	Animal	2016–1017	Italy, Spain, and Belgium	[55]
mcr-5	*Salmonella entérica*	bla_{TEM-1b}	4–8	Animal, food	2011–2013	Germany	[56]
mcr-6	*Moraxella pluranimalium*	bla_{BRO-1}	2–4	Animal	2014–2015	UK	[57]
mcr-7.1	*K. pneumoniae*	bla_{SHV-28}; $bla_{CTX-M-55}$	4	Animal	2014	China	[58]
mcr-8	*K. pneumoniae*	bla_{SHV-1}; bla_{NDM-1}; $bla_{CTX-M-14}$; bla_{NDM-5}	16	Animal	2015–2017	China	[59]
mcr-9	*Salmonella enterica* serovar Typhimurium	bla_{TEM-1B}; bla_{SHV-12}	5	Human	2010	USA	[60]
mcr-10	*Enterobacter roggenkampii*	bla_{MIR-5}	2	Human	2016	China	[61]

*Year of first gene isolation

**ND* not detected

to be reported in 2016 (Fig. 2). Carried by the IncX4 plasmid, the *mcr-1* gene was detected in *E. coli* strains (ST-101) recovered from a diabetic patient with a foot infection [20]. The case occurred in the Northeast Brazilian region and a complete genome analysis was performed [20].

In different studies, IncX4 has been identified as the main carrier of the colistin resistance gene in Enterobacteriaceae; these plasmids have been found in a variety of species, belonging to different "sequence types" (STs) and in a variety of samples, such as food, animals, and clinical isolates

Table 2 Relevant studies on the presence of the *mcr* gene in samples isolated from humans between 2016 and 2020 in Brazil

Gene	Year	City/Region	Sample	Specie	Plasmid	References
mcr-1	2016	Northeastern Brazilian region	Soft tissue	*E.coli* (ST-101)	IncX4	[62]
mcr-1.1	2015–2016	Santa Catarina	Blood	*E. coli* (ST-206, ST-354)	IncX4	[21]
mcr-1	2016	São Paulo	Blood	*E. coli* (ST-156)	IncX4	[22]
mcr-1 and *bla*KPC-2	2014	Porto Alegre	Rectal *Swab*	*K. pneumoniae* (ST-437)	IncX4	[66]
mcr-1	2016	Vitória	Urine	*K. pneumoniae* (ST-392)	IncX4	[69]
mcr-1 and *mcr*-5	2016	Recife	Blood	*Enterobacter*	IncX4 and Inc	[71]
mcr-4.3	2008	Brazil	Blood	*Acinetobacter baumannii* (597A)	pAb-MCR4.3	[72]
mcr-1	2016–2017	Paraná	Others	*K. pneumoniae* and *E. coli*	Undetermined	[73]
mcr-1	2017–2018	Santa Maria	Feces	Enterobacteria	Undetermined	[74]

Fig. 2 Distribution of *mcr* genes described in Brazil
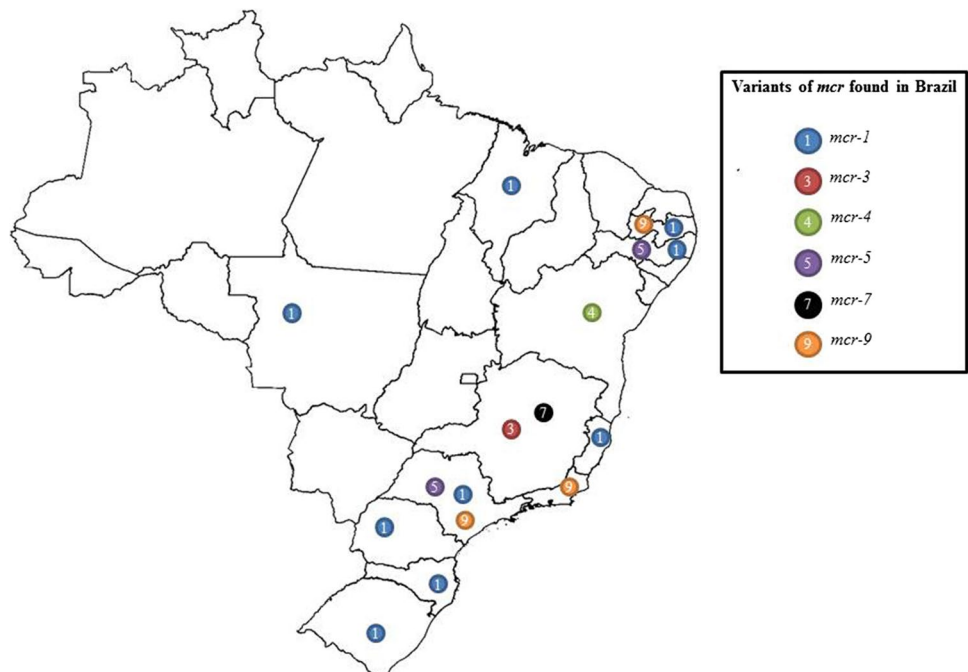
[20]. As a result, the presence of this carrier on different continents indicates that it is one of the most important factors behind the propagation of the *mcr-1* gene. Correspondingly, Zamparette et al. [21] reported the presence of IncX4 in two *Escherichia coli* isolates (ST-206 and ST-354) as a carrier plasmid for the *mcr-1.1* gene.

Analogously, Girardello et al. [64] isolated 490 colistin-resistant Gram-negative rods from human infections in a hospital setting in the Northeastern Brazilian region, of which eight were *mcr-1.1*-positive *Escherichia coli*. The genomes of these bacteria were sequenced, showing that *mcr-1.1* was located in a contig that is presumed to be part of an IncX4 plasmid in seven of eight isolates. In the last one, *mcr-1.1* was located in a contig that is presumed to be part of an IncHI2A plasmid.

Interestingly, isolates obtained from patients at an outpatient hospital in Santa Catarina (RS) who were not directly exposed to polymyxin showed an affinity of approximately 99.9% to the IncX4 plasmids reported in China and in Brazil for the first time. The IncX4 plasmid was found to be one of the main vectors of *mcr* spread in both analyses [21, 65]. Moreover, Vasconcelos et al. [67] coded the presence of other genes for MDR, such as *bla*CTX-M-2.

The coexistence of resistance genes has become a major concern due to the lack of therapeutic options [67]. Several studies have found the existence of more than one resistance gene in isolates of enterobacteria, especially in extended-spectrum β-lactamases (ESBL) producers [66]. Conceição-Neto et al. [68] and Dalmolin et al. [66] described the coexistence of colistin and carbapenem resistance genes.

Dalmolin et al. [66] reported a case of *K. pneumoniae* harboring *mcr-1* and bla_{KPC-2}. The analysis was carried out through the screening of 3468 isolates recovered in several hospitals in the Southern region of Brazil, between 2013 and 2016. Another case of *K. pneumoniae* carrying the *mcr-1* and bla_{KPC} genes was described in a patient diagnosed with thrombosis, in a hospital in Vitória (ES) [69]. In another study, a single isolate containing the two resistance genes was reported in Porto Alegre (RS). An analysis of the data revealed that the plasmids carrying *mcr-1* and bla_{KPC} belonged to the IncX4 and IncFIB groups, respectively [66].

Nitz et al. [70] assessed the molecular profile of virulence and resistance genes in 99 isolates of *P. aeruginosa* collected from various clinical specimens in São Luís (MA), one of which had the *mcr-1* gene. Except for carbapenems, imipenem, and meropenem, which it was sensitive to, this isolate showed resistance to at least one antibiotic from the following classes: ß-lactams, aminoglycosides, quinolones, and polymyxins, different from those presented in strains isolated from animal meat (Table 2).

Rocha et al. [70] showed the presence of *mcr-1* gene variants in isolates of *Enterobacteriaceae* recovered from clinical isolates in a hospital in Recife (PE). Multiple studies have indicated the presence of different variants in Brazil over the years in this context. Kieffer et al. [71], Martins-Sorenson et al. [72], and Dos Santos et al. [74] reported the emergence of *mcr-3.12*, *mcr-4.3*, and *mcr-3* and 7 alleles, respectively, carried by the IncY plasmid, in different types of samples and different isolates in South and Southeast regions of Brazil. These studies also showed a difference in the plasmids carrying the resistance genes. Kieffer et al. [71] described a plasmid IncA/C2, and Martins-Sorenson [72] described a previously unknown plasmid, pAb-MCR4,3, which carried mcr-4,3 in *A. baumannii* isolates. The *mcr-9.1* allele in *Salmonella typhimurium* isolates was first identified from a clinical isolate in 2019 collected in the Southern Brazilian region; however, due to rapid spread, this allele has been reported globally among *Enterobacteriaceae* [75].

In addition, the emergence of the COVID-19 pandemic, caused by the SARS-CoV-2 virus, expanded the number of antimicrobial interactions in hospitals, therefore causing an increase in the occurrence of secondary infections and the spread of microorganisms in this environment. The COVID-19 pandemic has also enhanced the frequent prescription of antibiotics by doctors, since most patients develop mild/moderate symptoms that resemble a bacterial infection at early stages, as well as the search for these drugs in pharmaceutical shops, contributing to the current challenge of antimicrobial resistance [76]. The study conducted by Polly et al. [77] in an acute care hospital in Brazil, for example, already describes an increase in the incidence of carbapenem-resistant *Acinetobacter baumannii* (CRAB) and methicillin-resistant *Staphylococcus aureus* (MRSA) infections both in ICUs and non-ICUs units after the pandemic. Thus, these factors may further favor the spread of strains carrying the *mcr* gene in human clinical isolates [78].

Incidence of the *mcr* gene in animal and animal meat

The extensive use of polymyxins in production animals was recognized as a major cause of the emergence and rapid spread of the resistance gene. In this context, the first reports of these isolates were obtained from pork and chicken retail fair trade [20, 65]. Vasconcelos et al. [67] identified IncX4 as a carrier of the *mcr-1* gene in strains of *E. coli* (ST-359) obtained from chicken carcasses from a public market in the Northeast Brazilian region.

Brazilian cattle without previous contact with polymyxin showed several resistance genes, according to Palmeira et al. [79]. In the study, the presence of *mcr-1* and $bla_{CTX-M-2}$ was observed in an *E. coli* isolate (ST-443) obtained in northeastern Brazil. Genes for colistin resistance and extended-spectrum β-lactamases (ESBL) were in separate plasmids, IncX4 and IncF, respectively. Fuentes-Castillo et al. [80], in turn, reported the coexistence of resistance genes in *Enterobacter kobei* (E11R) strains isolated in Angra dos Reis (RJ). The isolates were recovered from a culture of the respiratory exudate of a franciscana dolphin, an endangered species. The analyses reported the presence of *mcr-9.1* and $bla_{CTX-M-15}$, in addition to aminoglycoside, tetracycline, fluoroquinolone, fosfomycin, and sulfonamide resistance genes.

In 2020, *mcr-9*-mediated colistin resistance was described by Leite et al. [75] on *Salmonella typhimurium* (ST-19) isolates found in swine raised in the Northeastern Brazilian region. Resistance was determined by the minimum inhibitory concentration, and from genome sequencing, the presence of plasmids IncHI2 and IncHI2A was reported. *Salmonella enterica* serovar *Typhimurium* carrying the *mcr-1* was described in 2018, in samples taken from pork reservoirs in Southern Brazil [76]. In a study carried out by Rau et al. [81], *Salmonella enterica* carrying the colistin resistance gene was detected and characterized in foods produced in Brazil; 90% of the detected isolates belonged to the serovar *Typhimurium* and harbored the *mcr-1* gene in plasmid IncX4.

Studies carried out by Moreno et al. [82] detected another type of *Salmonella* carrying the colistin resistance gene. In the study, samples of chicken meat from São Paulo (SP) were selected for testing for the presence of the *mcr* gene. Two isolates that contained the gene were identified and genome analysis determined the presence of the serovar *Schwarzengrund* (ST-96) harbored in plasmid IncX4. The *Schwarzengrund* serovar had already been isolated from birds and in poultry meat; however, few studies indicated the

presence of bacterial resistance. Furthermore, the presence of *mcr* in fair trade samples and clinical isolates of enterobacteria has been frequently described. Saidenberg et al. [83] reported the presence of *mcr*-bearing strains of *E. coli* MDR (ST131-H22) in domestic poultry from São Paulo (SP). The isolates showed the *mcr-5.1* and *mcr-9* variants, suggesting the role of poultry farming as one of the main reservoirs of colistin resistance in pathogenic enterobacteria.

Correspondingly, Barbieri et al. [84] tested the presence of *mcr* genes in 107 *E. coli* isolates obtained from production poultry in Rio de Janeiro (RJ). The *mcr-1* gene was found in 62 (57.9%) isolates, while the *mcr-5* gene was discovered in 3 (2.8%) isolates; all of these isolates were phenotypically resistant to colistin (MIC > 8 μg/mL). IncI2, FIB, and B/O were the most prevalent replicon types discovered, accounting for 38, 36, and 34% of *mcr*-positive isolates, respectively.

A study conducted by Kobs et al. [85], demonstrated the presence of the polymyxin resistance gene in pets from the Brazilian state of Santa Catarina. In his analysis, this study identified the first *E. coli*, *Klebsiella* spp., and *Enterobacter* spp. isolates carrying *mcr-1* recovered from dogs and cats. A total of 64 bacterial isolates with phenotypic resistance to polymyxin B were identified, of which 62 isolates were from dogs and two from cats. Furthermore, all over the *mcr-1*-positive isolates were MDR. As a result, veterinary medicine should pay more attention to antimicrobial resistance.

Additionally, Ramos et al. [86] characterized 216 *E. coli* strains isolated from dogs fed either a raw meat-based (RMDB) diet or a conventional dry feed in Minas Gerais, southeastern Brazil. Isolates from RMBD-fed dogs were frequently positive for MDR *E. coli* isolates, and among them, seven ESBL producers were identified and subjected to whole-genome sequencing. The colistin-resistant gene *mcr-1* was found to be positive in two strains, ST57 and ST410. Moreover, a BLAST search of the nodes containing the ESBL and *mcr-1* genes revealed that the majority of them were found in mobile genetic components of varied replicon types, including the IncFII plasmid. Therefore, this study revealed that due to the risk of spreading these bacteria both within the household and in the community, ESBL and *mcr-1 E. coli* strains pose a potential risk not only to the host dogs but also to other animals and humans in the vicinity (Table 3).

Environmental incidence of the *mcr* gene

The existence of the *mcr* gene in pathogens that produce β-lactamases and carbapenemases, as shown in Table 4, is of concern for the scientific community [88]. Sacramento et al. [88] described the presence of the *mcr-1* and $bla_{\text{CTX-M-8}}$ genes in *E. coli* isolates recovered from a mangrove ecosystem in the Northeast region of Brazil. In this study, the

Table 3 Relevant studies on the presence of the *mcr* gene in samples isolated from clinical animals and retail meat between 2018 and 2020 in Brazil

Genes	Year*	City/region	Origin of the sample	Specie	Plasmid	References
mcr-1	2017–2018	Santa Catarina	Domestic animals (cat and dog)	*E. coli, Enterobacter* spp., *Klebsiella* spp. and *Pseudomonas* spp.	Undetermined	[85]
mcr-1	Not informed	Northeastern Brazilian region	Chicken carcass	*E. coli* (ST-359)	IncX4	[67]
mcr-9.1—$bla_{\text{CTX-M-15}}$	2019	Angra dos Reis	Dolphin respiratory exudate culture	*Enterobacter kobei* (E11R)	IncHI2—IncHI2A	[81
mcr-1—$bla_{\text{CTX-M-2}}$	2014	Northeastern Brazilian region	Bird and swine meat	*E. coli* (E12)	IncX4/IncF	[79]
mcr-9	2017	Southern Brazilian region	Swine samples	*S. typhimurium* (ST-19)	IncHI2, IncHI2A e pN1566-2	[75]
mcr-1	2014–2017	Brazil	Sample of retail poultry, turkey and pork meat, and carcass	*Salmonella entérica*	IncX4	[76]
mcr-1	2013–2016	São Paulo	Bird meat	*Salmomella enterica* serovar *Schwarzengrund* (ST-96)	IncX4	[82]
mcr-5.1 and *mcr*-9	2019	São Paulo	Domestic birds	*E. coli* (ST131-H22)	Undetermined	[83]
mcr-3.12	2018	Minas Gerais	Swine feces	*E. coli* (ST-641)	IncA/C$_2$	[71]
mcr-1	2019	Rio de Janeiro	Bird cloacal swabs	*Escherichia fergusonii*	IncHI2	[87]

*Year of first isolament

Table 4 Studies on the presence of the *mcr* gene in environmental samples between 2017 and 2019 in Brazil

Genes	Year	City/Region	Sample	Specie	Plasmid	References
mcr-1—*bla*$_{CTX-M-8}$	2017	Northeastern Brazilian region	Mangrove water	*E. coli* (ECEST9)	Incl1, IncX1, IncFII, IncFIB, and IncX4	[88]
mcr-1, *mcr*-3, and *mcr*-7	2019	Minas Gerais	Environmental feces samples (soil and water)	*Enterobacter*	IncY	[74]

presence of plasmids Incl1, IncX1, IncFII, IncFIB, and IncX4 was described. The colistin resistance gene was carried by the plasmid IncX4, corroborating the concept of this plasmid as one of the main agents of gene dissemination in enterobacteria [62]. Furthermore, the identification of pathogens with more than one resistance gene, especially those that present the *mcr-9.1* variant, has represented a sign of greater propagation of the *mcr* gene [80].

Cordeiro-Moura et al. [89] detected the presence of *mcr-1* in an *E. coli* strain isolated from a river sample in João Pessoa, Paraíba, as part of a project to analyze bacterial resistance in coastal waters. The strain isolated with the gene had an MIC of 4 μg/mL. In addition, plasmids IncX4, IncN, IncI1/Iγ, IncF, and IncFIB were identified in the isolate, in which the gene was identified as belonging to the IncX4 incompatibility group. Similarly, Fernandes et al. [65] detected the presence of the *mcr-1* gene mediated by plasmid IncX4 and *bla*$_{CTX-M}$ in *E. coli* strains isolated from coastal waters of the city of São Paulo (Table 4).

Final considerations

Due to its wide dissemination, bacterial resistance has become a challenge for the scientific community. Resistant Gram-negative bacilli are present mainly in clinical cases. Unfortunately, the development of effective drugs does not keep up with the prevalence rates of MDR strains, even antimicrobials that have high activity against Gram-negative strains, such as polymyxins, have become ineffective in the treatment of infections. This fact is aggravated by the lack of Brazilian laws that prohibit the use of antibiotics of this class as growth promoters in animals for consumption, considered one of the main sources of AMR dissemination. In this context, the present study highlights the importance of the One Health concept in controlling the spread of AMR, emphasizing the influence of animal health and the environment on the prevalence, and spread of bacterial resistance genes. The trajectory of the incidence of the *mcr* gene in Gram-negative bacteria can be used as an example of the need for studies that highlight and use the One Heath approach, in addition to the importance of molecular epidemiology in the control of bacterial resistance. Furthermore, the pandemic scenario that began in 2019 will possibly be a major accelerator of the spread of resistant bacteria in Brazil.

Data availability All data generated or analysed during this study are included in this published article.

Declarations

Consent to participate All authors approved the manuscript.

Consent for publication Written informed consent for publication was obtained from all participants.

Conflict of interest The authors declare no competing interests.

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