

# Resistance to polymyxins in *Escherichia coli*

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## ABSTRACT

**Introduction:** While in veterinary medicine polymyxins have never ceased to be used for the treatment of Enterobacteria infections, high resistance rates are observed in infections caused by Gram-negative bacteria producing carbapenems against colistin.

**Objective:** To review the aspects involved in resistance in *Escherichia coli* encoded by the gene *mcr-1*.

**Conclusion:** It is believed that this resistance, especially in *Escherichia coli*, is associated with the intense use of colistin in animal nurture, which represents an important public health problem.

**Key Words:** Microbial multiresistance; Gene *mcr-1*; Plasmids; Colistin

## INTRODUCTION

*Escherichia coli* belong to the family Enterobacteriaceae, represented by Gram-negative bacilli and are present in the enteric microbiota of humans and animals [1,2]. In certain circumstances, these bacteria are capable of causing various pathologies in both species. The most common infections caused by *E. coli* in humans are those of the urinary tract. These infections are usually associated with acute case and affect all ages. During the first year of life the infection occurs mainly in males due to the recurrence of urethral problems. While in adulthood, women present a higher incidence of this infection due to hormonal changes during pregnancy, menopause or during the fertile period [3,4]. Clinical conditions are variable, complications such as the development of cystitis and acute pyelonephritis, with renal and systemic insufficiency due to septicemia, if there is no adequate treatment [5,6].

Animal pathologies are related to several stress factors such as environmental management problems and malnutrition. In birds, *E. coli* samples that have virulence factors are designated as APEC (Avian pathogenic *E. coli*). The clinical manifestations are enteritis, hepatitis and involvement of organs such as kidneys, lung and bone marrow [7,8]. While in pig farming stands out enteric problems are related to diarrhea and in bovine culture the recurrence of mastitis caused by *E. coli* affects production and quality of milk, which also increases the final cost of this product, this being an infection that causes acute or chronic inflammations in the breasts of these animals [9-12].

The antibiotics used in the treatment of bacterial infections in veterinary and human medicine due mainly to the empirical mode of use, favor the selection of resistant strains, causing serious public health problems [13,14].

Polymyxins (A, B, C, D and E) form a group of polypeptide antibiotics synthesized by *Bacillus polymyxa* strains, active against Gram-negative bacteria, but only polymyxins B and E (colistin) are used clinically, while others have a high level of toxicity [15,16]. Studies conducted in the 1970s showed that colistin had nephrotoxic potential, resulting in the abandonment of this drug for medical therapy. However, the emergence of multiresistant Gram-negative bacilli has emerged the need to use colistin for the treatment of these infections. Thus, polymyxins B and E (colistin) had their use rescued at doses appropriate to the patient and through constant monitoring to control recurrent infections of Gram-negative species [17-19].

However, China's Ministry of Science and Technology recently described a new resistance gene to the antimicrobial colistin, the *mcr-1* gene that was isolated from *E. coli* strains. Colistin resistance in Gram-negative

microorganisms appears to be related to this gene, which is capable of encoding the enzyme transferase phosphoethanolamine, conferring catalytic modifications on the target of the colistin [20-21]. This finding is of great concern to the world scientific community, since colistin was until then the most potent antimicrobial used in the treatment of multiresistant infections caused by Enterobacteria [17-22].

In this context, the objective of this study was to approach the aspects involved in resistance in *E. coli* encoded by the *mcr-1* gene.

## METHOD

It was a systematic review of the main aspects involved in resistance in *E. coli* to colistin, mediated by the *mcr-1* gene, using data available in electronic banks, for the following descriptors: *mcr-1* gene; resistance in *E. coli*, multiresistance, colistin, polymyxins and Enterobacteria. Scientific articles included were published between the years of 2003 and 2017.

### Multiresistance

The increasing resistance rates observed in different microbial groups compared to the antibiotics that had previously been effective in the therapy of important infectious diseases seem to be related, in particular, to habits of inadequate use of these antimicrobials by the population, together with the empirical treatments that favor the selection of resistant isolates [23,24].

Antimicrobial resistance can occur due to spontaneous mutations or gene recombination, creating large genetic variability, overlapping natural selection, a condition in which the most suitable microorganisms have survival advantages. In addition, in non-chromosome resistance the resistant microorganism transmits this information to its progeny [25].

Although there are several antimicrobials that target is deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), in order to inhibit the synthesis of proteins, enzymes and components of the cell wall, spontaneous chromosomal resistances can occur, where the bacterium exchanges a nucleotide or modifies the synthesis of proteins, making it difficult to find the antimicrobial with its target [26]. There is also plasmid-mediated resistance, where the transfer of information from one resistant microorganism to another sensitive microorganism, being possible to occur between microorganisms of the same species and between different species, conferring resistance by autonomous replication of plasmids [27].

### Resistance in Enterobacteria

There are several mechanisms of resistance related to this bacterial group,

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among which the inactivation of the drug by enzymes, modification of the target of action of the antimicrobial, efflux pumps, and decrease in drug capture by membrane modification and blockade since most antibiotics are produced to cross the bacterial membrane and achieve it is target such as, for example, quinolones [28,29].

One of the most relevant mechanisms of plasmid-mediated resistance in Gram-negative bacteria is the production of  $\beta$ -lactamase enzymes which are capable of binding to the carbonic portion of the  $\beta$ -lactam ring of the antimicrobial by covalent bond, hydrolyzing the amide groups of the structure, and production of amplified spectrum  $\beta$ -lactamases (ESBL) [30,31].

Carbapenems are antibiotics widely used in Enterobacteriaceae infections, with meropenem, imipenem, doripenem and ertapenem being the most used in clinical practice. However, resistant strains also to this important class of antimicrobials have provoked hospital outbreaks and frequent worldwide, due to the indiscriminate use and the adaptive response of the bacteria [32].

Resistance to carbapenems was identified in *Klebsiella pneumoniae*, being able to degrade  $\beta$ -lactam drugs by enzymes carbapenemases called KPC. These plasmid-encoded enzymes are divided according to the dependence of ions, with classes A, C and D being serine  $\beta$ -lactamases independent of zinc, while class B enzymes are zinc-dependent metallo-beta-lactamases (MBLS) for activation [33,34].

The New Delhi enzyme or metallo-beta-lactamase (NDM) was first reported in the city of Sweden in 2009 by a traveler who was hospitalized in India, found in *K. pneumoniae*, the most recent finding being a new enzyme against  $\beta$ -lactam antibiotics. There are currently described 13 variants of the NDM enzyme, classified as NDM-1 through NDM-14, but to NDM-11 there is no assignment as a variant of the enzyme [35-36].

Cephalosporins are broad spectrum antibiotics, however, over time various forms of multidrug resistance have emerged. Ceftazidime is a third generation cephalosporin used since the decade of 1980 because of its activity against Gram-negative and Gram-positive bacteria. However, various mechanisms of bacterial resistance, such as NDM, ESBL, KPCs and chromosomal  $\beta$ -lactamases, led to resistance to cephalosporin [37].

Despite the existence of several classes of antibiotics, the high resistance rates observed have forced, as a last alternative against infections by Gram-negative bacteria, the use of polymyxins as colistin, due to the therapeutic efficacy of this against these bacteria [38].

### Polymyxins

Polymyxins are polypeptide antimicrobials discovered in 1950 and isolated from the *Bacillus* sp. culture, present in the soil. Polymyxins have five classes (A, B, C, D and E), but only two of these have a therapeutic action. Polymyxin B isolated from the *Bacillus polymyxa* species and polymyxin E or colistin, isolated from the microorganism *Bacillus colistinus*, configuring two antibiotics used in clinical practice against multiresistant Gram-negative bacteria. These polymyxins are differentiated by chemical structure, with polymyxin B having a D-phenylalanine amino acid at position six, while colistin has a D-leucine molecule in the same position [39,40].

Polymyxin B is a chemical compound in the form of sulfate, capable of dissociation in aqueous media or blood and in lipid membranes, since it is an amphipathic antibiotic. While colistin is a prodrug used in the form of sodium colistimethate, being transformed, after metabolism, into colistin base [19].

The mechanism of action of polymyxins is to act on the cell membranes and cytoplasm of bacteria. The antibiotic binds in the cellular envelope such as in the phospholipids and lipopolysaccharides and remove calcium and magnesium molecules that stabilize the membrane, causing the increase of the permeability of the same, leading to the loss of the cellular components and consequently to the cellular death [41].

Mechanisms of resistance to polymyxins are characterized by two phenotypes, the first one, called natural or intrinsic resistance, which is probably related to the result of a mutation in the bacterial genome, with levels of resistance at the minimum inhibitory concentration (MIC) of the antibiotic, [42], second, called the adaptive mechanism, occurs when bacteria after contact with high antimicrobial concentration are selected, that is, in increasing concentrations of polymyxins. This mechanism can select strains with very high MIC, which can be reversed in the absence of the selective pressure established by the drug [43].

The main resistance mechanism of Gram-negative bacteria to polymyxins is associated with the change in lipopolysaccharide lipid A (LPS), which is one of the major structural components of the bacterial cell wall [41].

The mechanism of resistance involving total inactivation of the lipid A biosynthetic protein, which may occur through different events, such as low magnesium and high calcium concentrations, changes in pH, presence of iron, deletions and mutations, occurring from inactivation of the first three genes of lipid A (IpxA, IpxC e IpxD) [19].

The development of gene resistance is due to two components used by several bacterial species to develop resistance and pathogenicity. The first component is related to the gene regulation that is due to a histidine kinase sensing protein that executes environmental stimuli and thus performs autophosphorylation, activating a cytoplasmic protein that causes transphosphorylation. This protein, in turn, activates or represses target genes, triggering resistance to polymyxins [41]. The second component was studied by Beceiro et al. [44] from the isolates of *Acinetobacter baumannii*, where they observed that the resistance can be also mediated by the mutation of the PmrA and PmrB genes, demonstrating that the mutation or increase of the expression of these genes leads to the addition of phosphoethanolamine in the lipid A, reducing the negative charge of this and consequently the action of polymyxins.

The first study related to the involvement of lipid A alterations in bacteria was carried out in *Salmonella enterica*, later other studies related to this same mechanism were described, from *Salmonella typhimurium*, *Yersinia pestis* and *Escherichia coli* [45].

Navarre et al. [46] reported that low concentrations of magnesium activated the PhoPQ system, whose purpose is to modify cell signals in response to environmental changes, promoting the expression of *pmrD* gene, while in *Klebsiella pneumoniae* isolates observed changes in the PhoPQ and PmrAB systems, developing resistance to polymyxins, due to modifications of the lipid A of the LPS of the bacteria from the mutation or inactivation of genes.

Arena et al. [47] and Jayol et al. [48] studying colistin-resistant *Klebsiella pneumoniae* isolates, have noted that a mechanism of colistin resistance may occur when loss of MgrB protein function occurs, affecting several biochemical pathways that are regulated by the transduction of PhoP-PhoQ signals, following modifications in LPS.

In addition to these mechanisms, the first case of resistance to plasmid-mediated colistin encoded by the *mcr-1* gene in China [49] has recently been reported, bringing the attention of the scientific community to investigate this form of resistance in other countries. Since, a growing number of Enterobacteria isolates in humans, animals and the environment have been studied and related to this form of resistance [50]. Until now, the *mcr-1* gene is known to be present in the following bacterial species: *E. coli*, *Salmonella enterica*, *K. pneumoniae*, *Enterobacter aerogenes* and *E. cloacae* [44].

Hinchliffe et al. [51] reported that the mechanism of plasmid-mediated colistin resistance and the *mcr-1* gene leads to changes in the target of colistin through the action of the enzyme phosphoethanolamine transferase, which transfers the glucosamine from lipid A, in this way, the negative charge of lipid A is reduced and, consequently, colistin can not bind. The same study showed that the activity of the *mcr-1* gene depends on the formation of zinc and disulfide bonds.

The major concern attributed to the *mcr-1* gene is that it has the ability to perform other arrangements in plasmids, allowing the dissemination of these among microorganisms present in animals and humans [52]. In addition, this type of resistance may be present in other resistance genes to different drugs, such as carbapenem and  $\beta$ -lactams [53]. Several strains of *E. coli* isolated from chicken meat had resistance to colistin by the *mcr-1* gene and also the presence of the NDM gene and variants, generating hybrid plasmids. There were also reports of patients with urinary tract infection in the USA who presented *blaKPC-2* gene together with *mcr-1* [54].

Xavier et al. [55] also found a new gene conferring resistance to colistin, the *mcr-2* gene, to date, isolated only in *E. coli*, also plasmid-mediated, present in fecal samples of 53 pigs in Belgium. The study found that this gene encodes phosphoethanolamine transferase with a higher prevalence than *mcr-1*. The *mcr-2* gene has 1626 bp and 76.7% of nucleotides identical to the *mcr-1* gene, whereas the *mcr-1* gene has a lower base, respectively 1617 bp. The distribution of *mcr-2* in countries European and *mcr-1* Japan may be related to geography or even to veterinary practices among these countries [56]. The high prevalence of the *mcr-1* gene in *E. coli* isolates from animal meat origins, mainly the pig meat in 2014, representing a percentage of 20%, respectively

of contaminated animals in China, while patients presented a percentage of 0 to 7% in *Klebsiella pneumoniae* isolates [20].

#### Final considerations

In this context, polymyxins, especially colistin, considered one of the last therapeutic options available for treatment of infections by multiresistant enterobacteria, are no longer effective, reflecting an alarming public health problem. The different mechanisms of resistance to polymyxins, especially the recent description of the *mcr-1* and *mcr-2* genes mediated by plasmids, especially in *E. coli*, is due to the importance of the implementation of preventive measures and the reduction of the consumption of antibiotics in the production of meats. In addition to highlighting the importance of simple attitudes, such as cleaning and disinfection of breeding sites, thus contributing to the reduction of the spread of resistant bacteria and, consequently, reduction of the use of drugs to treat diseases caused by these microorganisms.

This selection of colistin-resistant bacteria raises major concerns about the treatment of Gram-negative bacteria infections, so the USA Food and Drug Administration (FDA) intervened in 2017 through a new policy on the use of medicinal products for animals, and has banned the use of human antimicrobials, in creating these [57].

Providing access to guidelines on the importance of the rational use of antibiotics in human beings and veterinary medicine, besides promoting adequate sanitation and immunization policies, are actions that will contribute to the worldwide prevention of infections and, especially, to the reduction of resistant microorganisms.

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